Science: Opinion Fiction or Reality?

OPCUG & PATACS

January 15, 2022

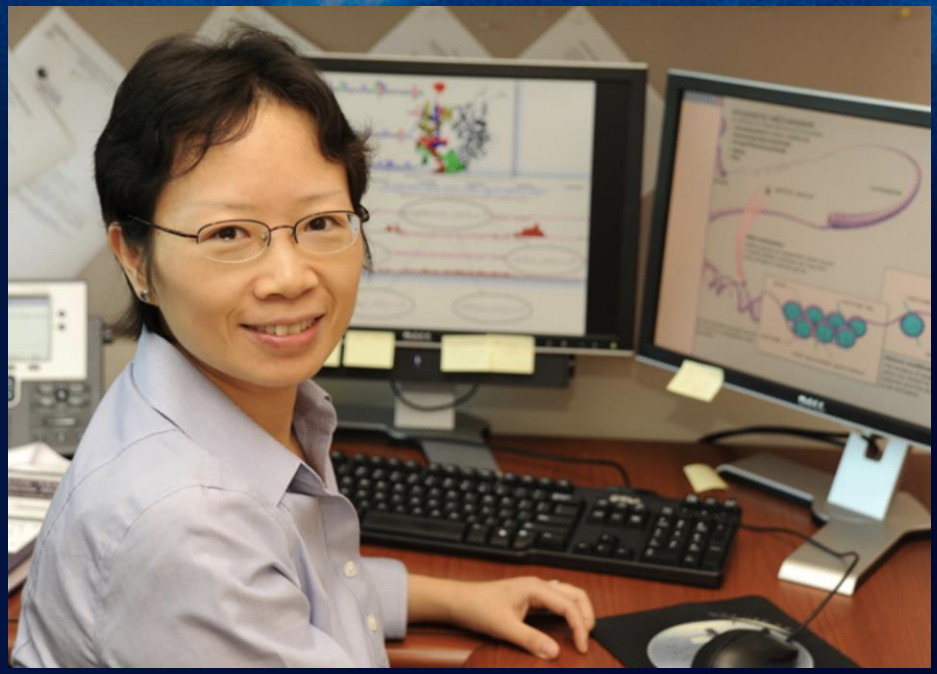
Lorrin R. Garson

Conclusion: is Science…

- Just opinion? **No**—not unless explicitly stated
- Fiction? **No**—unless it's in the genre "science fiction"
- Reality? **Yes**

Now here's why…

Chemistry as Exemplar of Science Information (more broadly STEM*)



1960s → today

* STEM = **S**cience, **T**echnology, **E**ngineering and **M**edicine PLUS Mathematics

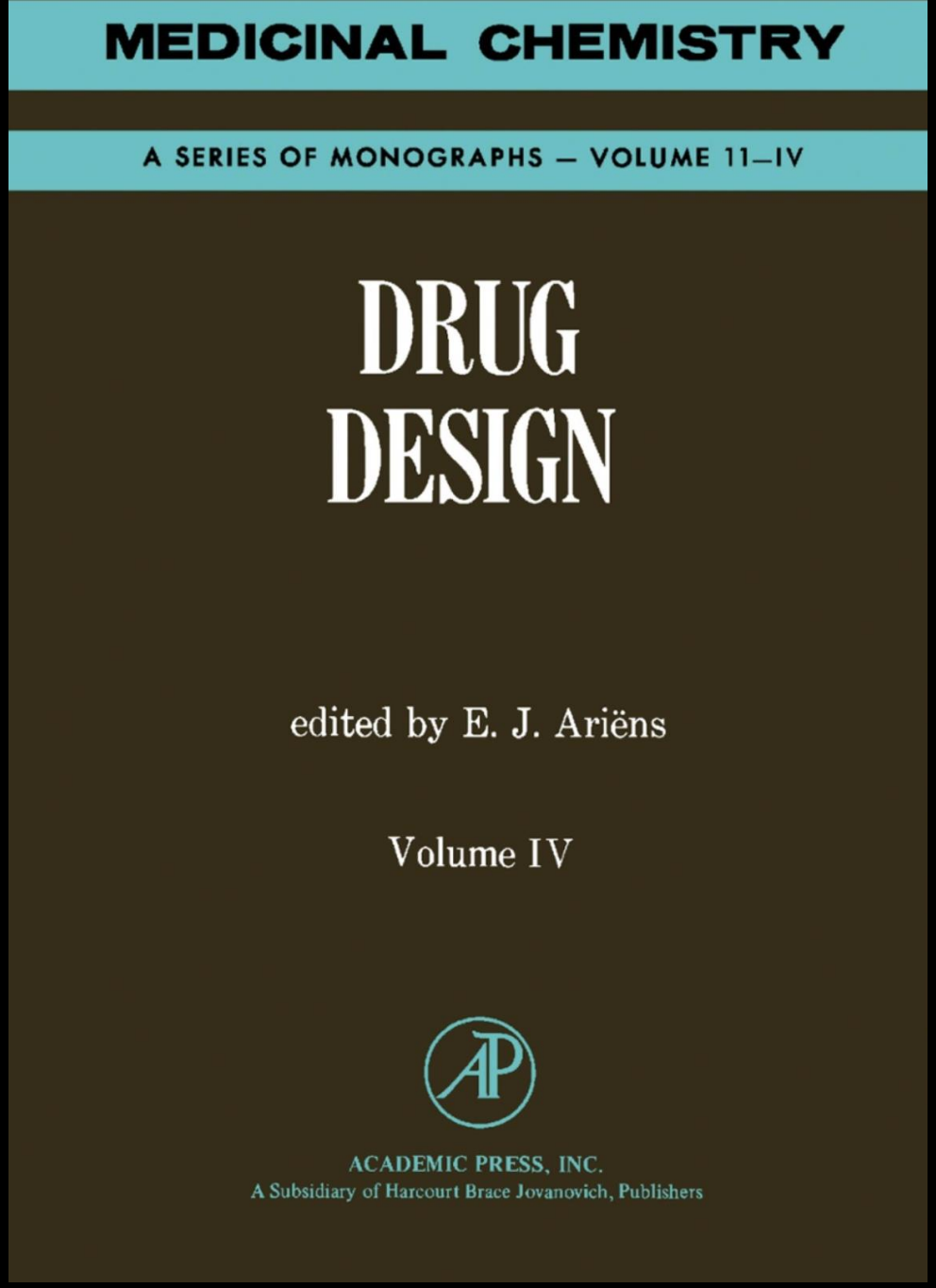
Sources of Science* Information

- Magazines
  - ✓ *Popular Science*
  - ✓ *Scientific American*
  - ✓ *Science* (aka *Science Magazine*)
  - ✓ *Chemical & Engineering News*
- Books
  - ✓ textbooks
  - ✓ science monographs
  - ✓ handbooks

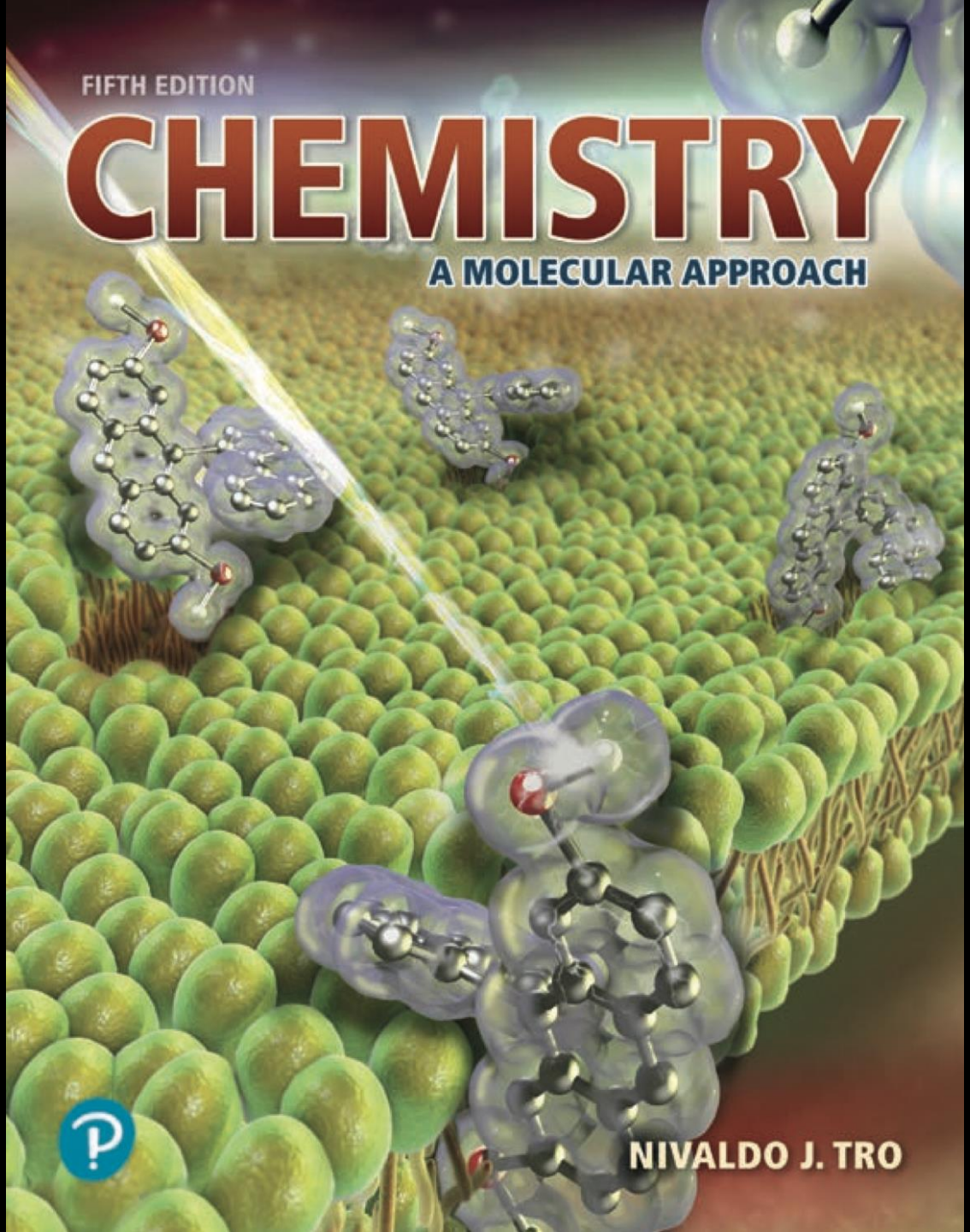
* In this presentation “science” and STEM will be used interchangeably

Magazine and Journal

Everhardus Jacobus Ariëns, M.D., Ph.D.

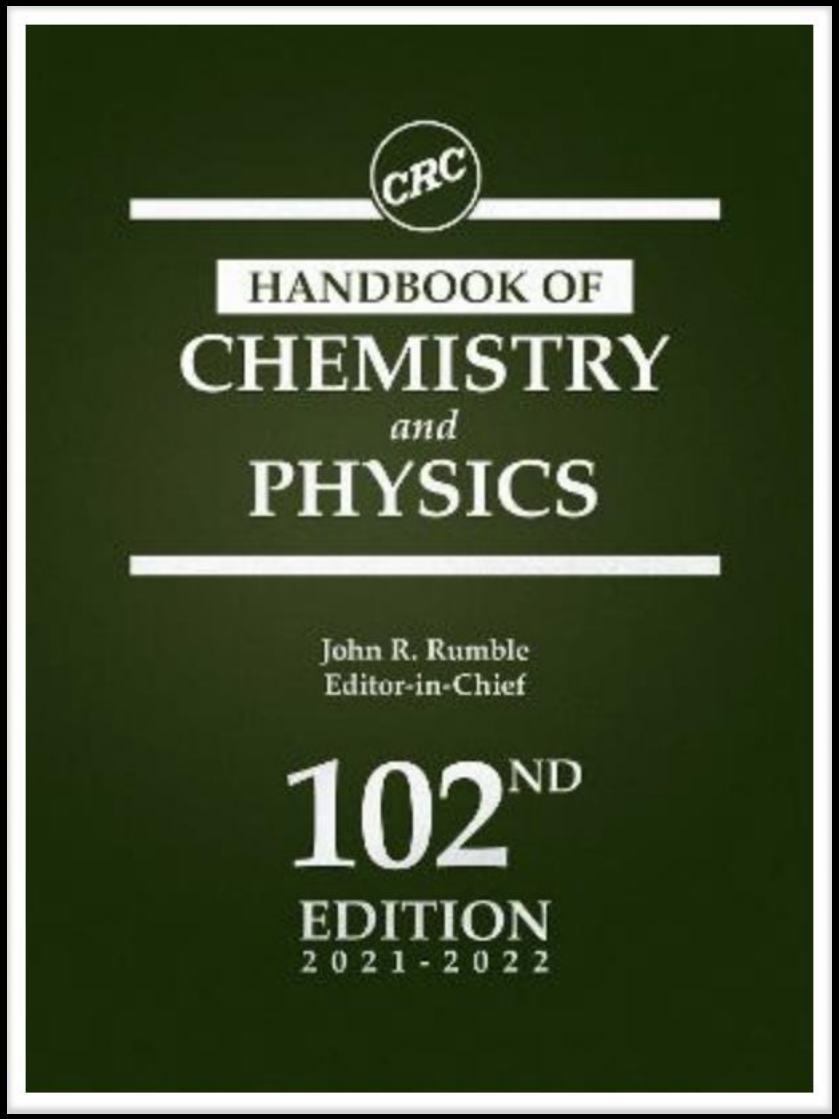


1323 pp.
$227


FIFTH EDITION
CHEMISTRY
A MOLECULAR APPROACH
NIVALDO J. TRO

1624 pp
~$200

1st Edition
1914



Also available electronically see ← Active hypertext link

Sources of Science Information (cont.)

- Abstracts and indexes
  - ✓ *Biological Abstracts*
  - ✓ *BIOSIS*
  - ✓ Chemical Abstracts Service*
  - ✓ *Index Medicus/Medline*
  - ✓ *PubMed*

* A division of the American Chemical Society

Chemical Abstracts Service*

- Approximately 1,400 employees
- Covers ~8,000 journals plus technical reports, dissertations and conference proceedings
- Patents from 27 countries and two international organizations
- In 50 languages
- For more information see 

* Established in 1907

Sources of Science Information (cont.)

- Patents
  - ✓ international in scope
  - ✓ primarily legal documents

United States Patent **3,668,226**
Quintana , et al. **June 6, 1972**

STRAIGHT CHAIN ALIPHATIC CARBOXYLIC ACID MONOESTERS OF 1,3-DIHYDROXY-2-PROPANONE

Abstract

This invention relates to perdurable insect repellent compositions comprig a precursor molecule which includes a moiety with dermal anchoring properties linked to a moiety with insect repellent properties. There are also described six new compounds, illustrative of the perdurable insect repellent structure. The six compounds are monoesters of 1,3-dihydroxy-2-propanone and the straight-chain aliphatic carboxylic acids--propanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, and undecanoic acid.

Inventors: **Quintana; Ronald P.** (Memphis, TN), **Garson; Lorrin R.** (Memphis, TN), **Lasslo; Andrew** (Memphis, TN)
Assignee: **The United States of America as represented by the Secretary of the Army** (N/A)
Family ID: **21765359**
Appl. No.: **05/014,417**
Filed: **February 26, 1970**

Current U.S. Class:	**554/227**; 514/919; 560/264; 549/379
Current CPC Class:	C07C 69/28 (20130101); A01N 37/12 (20130101); Y10S 514/919 (20130101)
Current International Class:	A01N 37/12 (20060101); C07C 69/00 (20060101); C07C 69/28 (20060101); C07c 069/28 (); A01n 009/24 ()
Field of Search:	;260/410.6,488J

Sources of Science Information (cont.)

- Conferences and congresses
- Internal seminars
- Information exchange between individuals

Science Journals—the Primary Source

- The established record of science
- Contain articles written by active scientists (not journalists)
- First science journal published in 1665
  - ✓ *Journal des sçavans*
  - ✓ *Philosophical Transactions of the Royal Society*
- **Peer reviewed**
- For more information see

Who Publishes Science Information?

- Commercial publishers (~90-95% of chemistry)
  - ✓ Elsevier
  - ✓ John Wiley
  - ✓ Springer/Macmillian
- Not-for-profit publishers (~5-10% of chemistry)
  - ✓ American Chemical Society
  - ✓ Gesellschaft Deutscher Chemiker
  - ✓ Royal Society of Chemistry
- For lists of science journals see

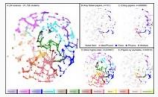
Journal ranking

From Wikipedia, the free encyclopedia

Journal ranking is widely used in academic circles in the evaluation of an academic journal's impact and quality. Journal rankings are intended to reflect the place of a journal within its field, the relative difficulty of being published in that journal, and the prestige associated with it. They have been introduced as official research evaluation tools in several countries.

Are some science journals more prestigious than others?

Part of a series on
Citation metrics



Altmetrics · Article-level · Author-level (Eigenfactor · G-index · H-index) · Bibliographic coupling · Citation (Analysis · Dynamics · Index · Graph) · Co-citation (Proximity Analysis) · Coercive citation · I4OC · **Journal-level** · Kardashian Index
V · T · E

Measures [edit]

Traditionally, journal ranking "measures" or evaluations have been provided simply through institutional lists established by academic leaders or through committee vote. These approaches have been notoriously politicized and inaccurate reflections of actual prestige and quality, as they would often reflect the biases and personal career objectives of those involved in ranking the journals; also causing the problem of highly disparate evaluations across institutions.[1][2] Consequently, many institutions have required external sources of evaluation of journal quality. The traditional approach here has been through surveys of leading academics in a given field, but this approach too has potential for bias, though not as profound as that seen with institution-generated lists.[2] Consequently, governments, institutions, and leaders in scientometric research have turned to a litany of observed bibliometric measures on the journal-level that can be used as surrogates for quality and thus eliminate the need for subjective assessment.[1]

Consequently, several journal-level metrics have been proposed, most citation-based:

- Impact factor and CiteScore – reflecting the average number of citations to articles published in science and social science journals.
- SCImago Journal Rank – a measure of scientific influence of scholarly journals that accounts for both the number of citations received by a journal and the importance or prestige of the journals where such citations come from.
- *h*-index – usually used as a measure of scientific productivity and the scientific impact of an individual scientist, but can also be used to rank journals.
  - h5-index – this metric, calculated and released by Google Scholar, is based on the h-index of all articles published in a given journal in the last five years.[3]
- Expert survey – a score reflecting the overall quality or contribution of a journal is based on the results of the survey of active field researchers, practitioners and students (i.e., actual journal contributors or readers), who rank each journal based on specific criteria.[4]
- Publication power approach (PPA) – the ranking position of each journal is based on the actual publishing behavior of leading tenured academics over an extended time period. As such, the journal's ranking position reflects the frequency at which these scholars published their articles in this journal.[5][6]

Prestigious Car Brands

Prestigious Clothing Brands

Prestigious Watch Brands

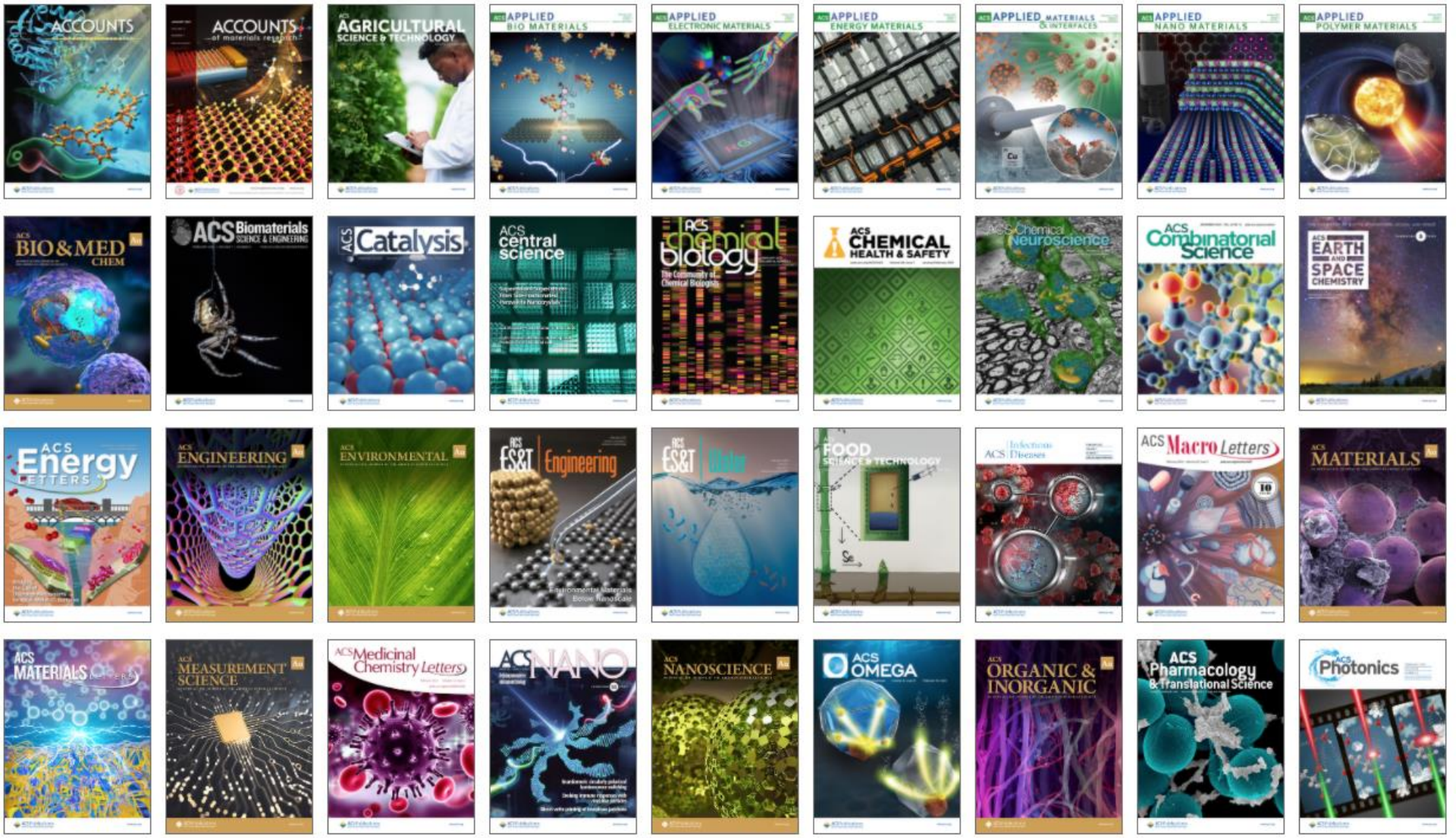
Prestigious Medical Journals

Top 15 Chemistry Journals

Rank	Title	Publisher
1	*Chemical Reviews*	American Chemical Society
2	*Journal of the American Chemical Society*	American Chemical Society
3	*Angewandte Chemie—International Edition*	John Wiley & Sons
4	*Chemical Society Reviews*	Royal Society of Chemistry
5	*Nano Letters*	American Chemical Society
6	*Nature Materials*	Nature Publishing Group
7	*Accounts of Chemical Research*	American Chemical Society
8	*Environmental Science & Technology*	American Chemical Society
9	*Chemistry of Materials*	American Chemical Society
10	*Chemical Communications*	Royal Society of Chemistry
11	*Journal of Agricultural and Food Chemistry*	American Chemical Society
12	*Nature Communications*	Nature Publishing Group
13	*Carbon*	Elsevier
14	*Chemistry—A European Journal*	Wiley-VCH
15	*Chemosphere*	Elsevier

See also

75 ACS Journals



75 ACS Journals

1879

Science & Engineering—Published Papers*

Rank	Region, country, or economy	2008	2018
-	World	1,755,850	2,555,959
1	China	249,049	528,263
2	United States	393,979	422,808
3	India	48,998	135,788
4	Germany	91,904	104,396
5	Japan	108,241	98,793
6	United Kingdom	91,358	97,681
7	Russia	31,798	81,579
8	Italy	56,157	71,240
9	South Korea	44,094	66,376
10	France	66,460	66,352
11	Brazil	35,490	60,148
12	Canada	53,296	59,968
13	Spain	44,191	54,537
14	Australia	37,174	53,610
15	Iran	17,034	48,306
-	EU	528,938	622,125

EU = European Union.

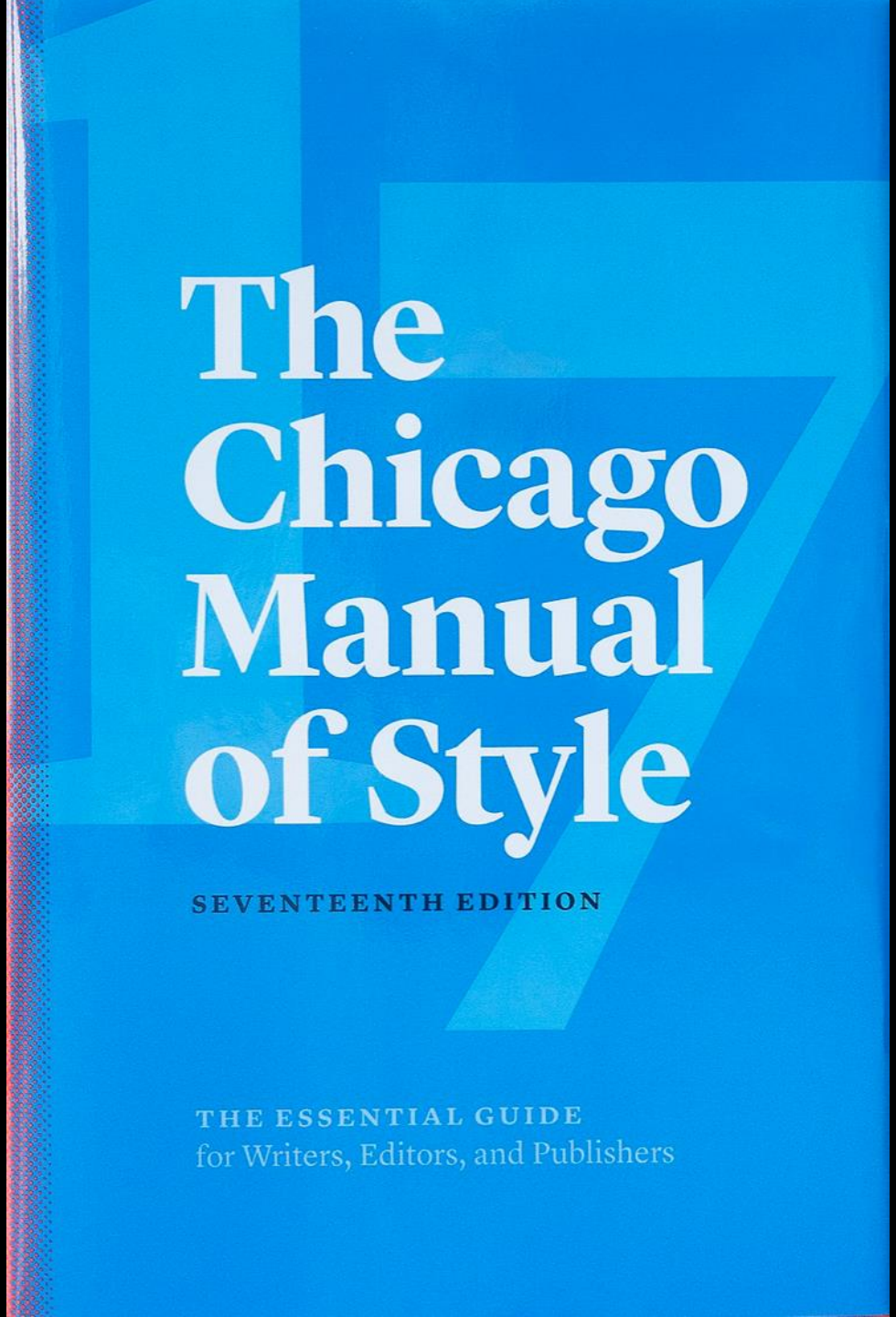
← 45% growth

* From the National Science Foundation

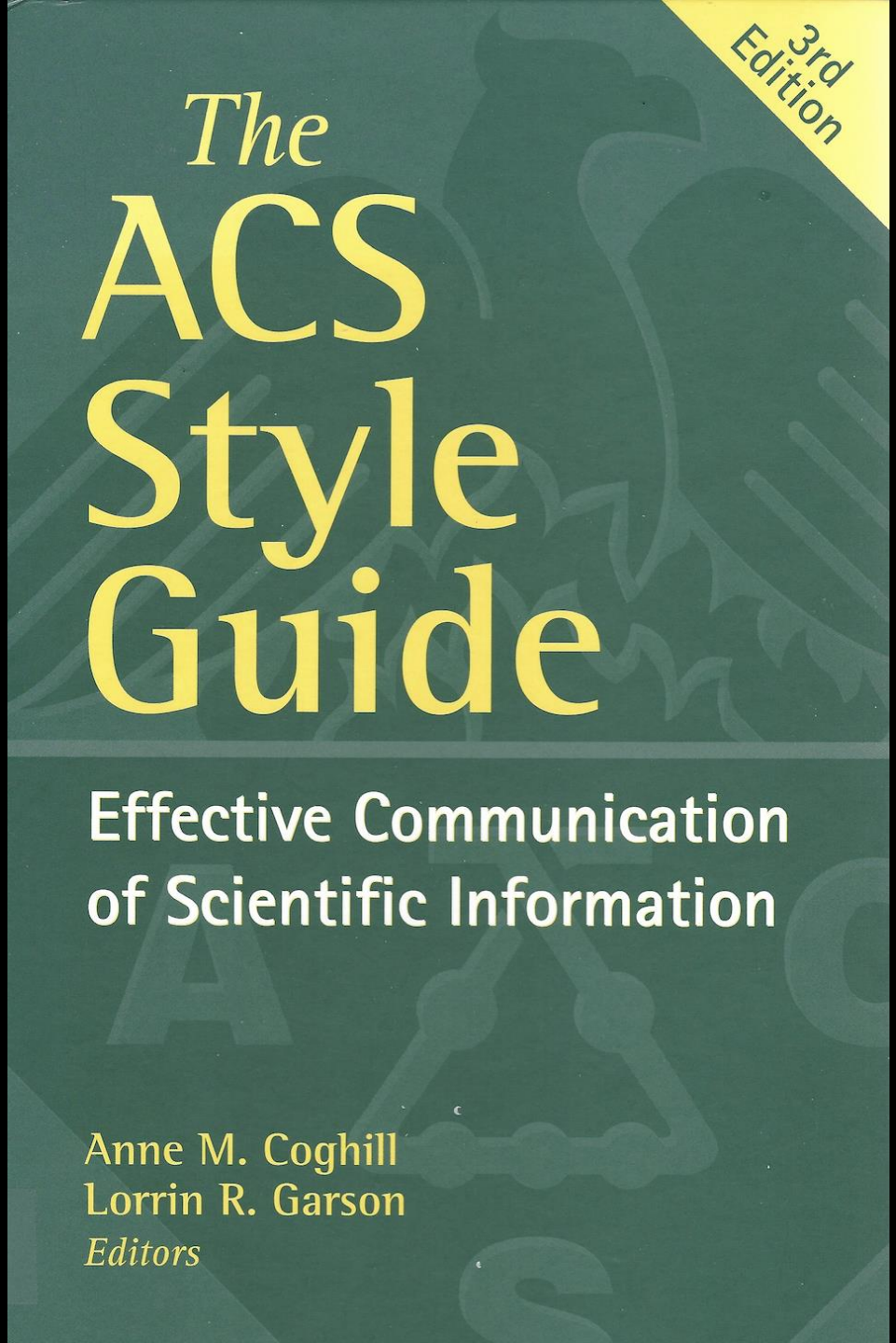
So… How Does Science Get Published?

2017
Edition

1st Edition
1906



2006 Edition



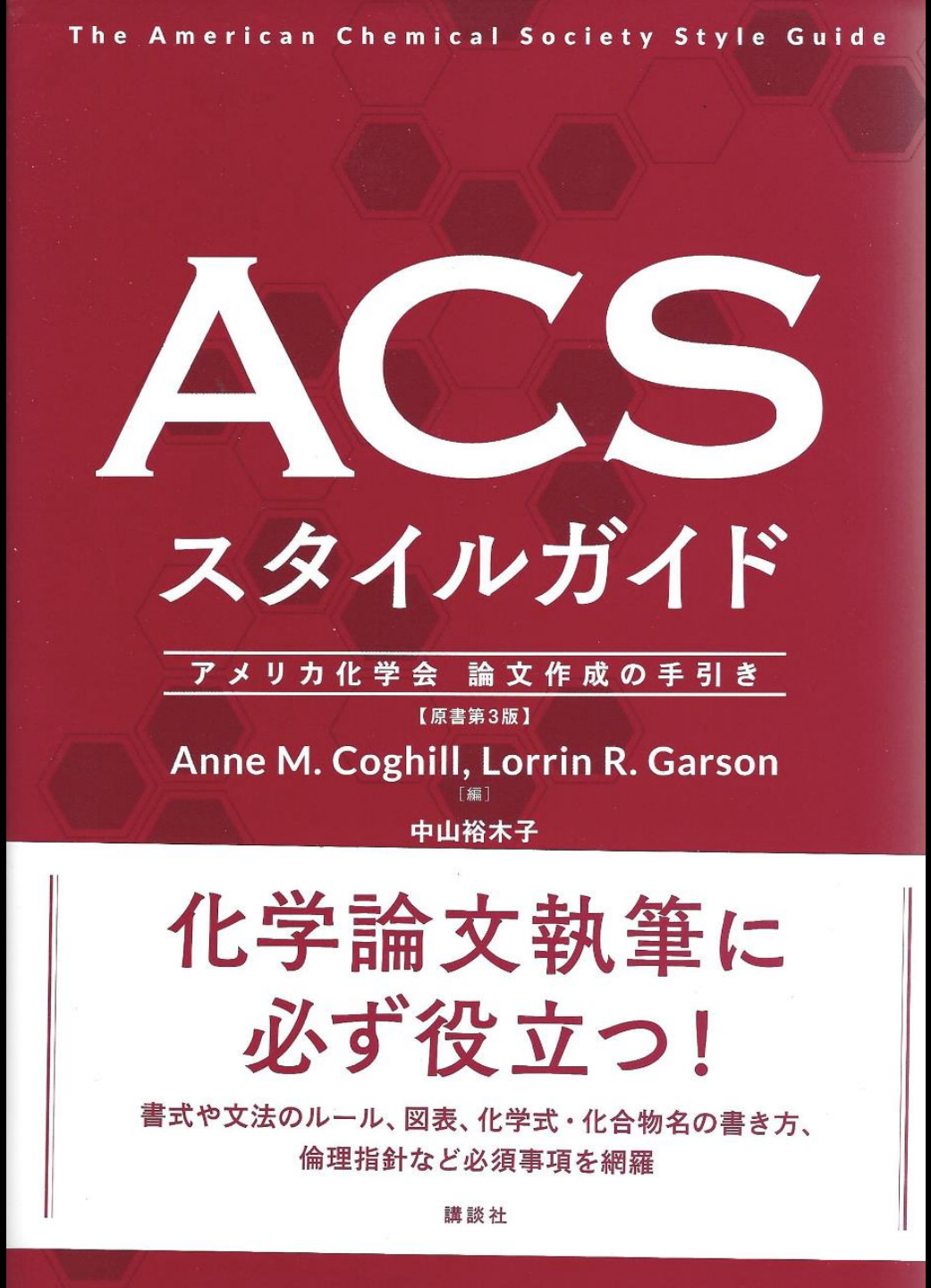
(ACS Style Guide)

Contents

(ACS Style Guide)

Part 2. Style Guidelines

2019
Edition



(ACS Style Guide)

It's all ~~Greek~~ Japanese to me!

目次

Manuscript Submission Process

- Manuscripts are written when something new or important to report*
- Authors select appropriate and most prestigious journal
- Manuscript is to be submitted to only one journal at a time

*** Publish or Perish!!**

Manuscript Review

- Manuscript read by editor-in-chief or associate editor*
- Editor accepts for consideration or rejects outright
- Editor selects 2-6 reviewers**

* *JACS* has 30 editors 

** Reviewers are not identified to authors

Manuscript Review: Peer Reviewers

- Practicing scientists with expertise in the field relevant to the manuscript
- Reviewers carefully scrutinize manuscripts
- Reviewers comment on:
  - ✓ deficiencies in the science
  - ✓ correct minor errors and style
  - ✓ suggestion further work if needed

Peer Reviewers (cont.)

- Reviewers report to editor to conditionally accept or reject
- Editor communicates with the author

Communication Cycle

- Reviewer ↔ Editor ↔ Author
- If manuscript to be published everyone is to be satisfied (usually)…
  - ✓ editor
  - ✓ reviewers
  - ✓ author
- Journal editor makes the final decision whether to publish

Work of production editor

Communicated to author

Edit Trace of Manuscript 10.1021/acs.langmuir.6b03367

Competitive and ~~synergistic interactions~~Synergistic Interactions between ~~polymer micelles~~Polymer Micelles, ~~drugs~~Drugs, and ~~cyclodextrins~~Cyclodextrins: ~~the importance~~The Importance of ~~drug solubilisation locus~~Drug Solubilization Locus

Author, please respond to the following questions:

Author: If references are cited in the Abstract, the full reference must be given. The Abstract will be available online separate from the main article, and the citations will not link to the reference list, making the citations appear out of context. 2

Author: Please verify whether the labels for footnotes b and c are in the correct locations in Table 3 8

Author: Please check that the citation for ref 20 is correct. What does "D. p. u. N. a." mean? 22

Margarita **Valero** ,[*,a,†] ■ Franca **Castiglione** ,[b,‡] ■ Andrea **Mele** ,[b,‡] ■ Marcelo A. **da Silva** ,[c,§] ■ ■ I. **Grillo** , ■ Gustavo **González-Gaitano** ,[d,||] ■ **and** Cécile A. **Dreiss** [*,c,§] ■

[a] **Dpto. Química Física, Facultad de Farmacia, Universidad de Salamanca** , Campus Miguel de Unamuno, s/n, 37007 Salamanca, Spain

[b] **Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano** , Piazza L. Da Vinci 32 ‖ I-20133 ~~,~~ Milano, Italy

[c] **Institute of Pharmaceutical Science, King's College London** ■ , Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, ~~U.K.~~ United Kingdom

[d] **Departamento de Química, Universidad de Navarra** , 31080 Pamplona, Spain

* ~~Corresponding author~~E-mail: mvalero@usal.es ~~; cecile.dreiss@kcl.ac.uk~~

† E-mail: cecile.dreiss@kcl.ac.uk.

Polymeric micelles, in particular PEO-PPO-based Pluronic, have emerged as promising drug carriers, while cyclodextrins, cyclic oligosaccharides with an apolar cavity, have long been used for their capacity to form inclusion complexes with drugs. Dimethylated β-cyclodextrin (CD) has the capacity to fully ~~break up~~breakup F127 Pluronic micelles, while this effect is substantially hindered if drugs are loaded within the micellar aggregates. Four drugs were studied at physiological temperature: lidocaine (LD), pentobarbital sodium salt (PB), sodium naproxen (NP), and sodium salicylate (SAL); higher temperatures shift the equilibrium ~~towards~~toward higher drug partitioning and lower ~~drug:CD~~drug/CD binding compared to 25 °C (Valero, M.; Dreiss, C. A. Growth, Shrinking, and Breaking of Pluronic Micelles in the Presence of Drugs and/or β-Cyclodextrin, a Study by Small-Angle Neutron Scattering and Fluorescence Spectroscopy. *Langmuir* **2010**, *26*, 10561−10571). ~~+~~ The impact of drugs on micellar structure was ~~characterised~~characterized by small-angle neutron scattering (SANS), while their ~~solubilisation~~solubilization locus was revealed by 2D NOESY NMR. UV and fluorescence spectroscopy, Dynamic and Static Light Scattering were employed to measure a range of micellar properties and drug:CD interactions: binding constant, drug partitioning within the micelles, critical micellar concentration of the loaded micelles, aggregation number (N_{agg}). Critically, time-resolved SANS (TR-SANS) reveal that micellar ~~break up~~breakup in the presence of drugs is substantially slower (100s of seconds) than for the free micelles (< 100 ms) (Valero, M.; Grillo,

What Does a Published Article Look Like?

- Sample article: *J. Med. Chem* 2021, **64**, 16425-16449
- Note: Article is 25 pages in length
- Note: Over 16,000 pages published in this journal as of October 29, 2021

Journal name ➔

Journal of Medicinal Chemistry

pubs.acs.org/jmc

Featured Article

Article title ➔

Discovery of Modified Amidate (ProTide) Prodrugs of Tenofovir with Enhanced Antiviral Properties

Authors ➔

Filip Kalčic, Michala Zgarbová, Jan Hodek, Karel Chalupský, Martin Dračínský, Alexandra Dvořáková, Timotej Strmeň, Jaroslav Šebestík, Ondřej Baszczyński, Jan Weber, Helena Mertlíková-Kaiserová, and Zlatko Janeba*

Citation ➔

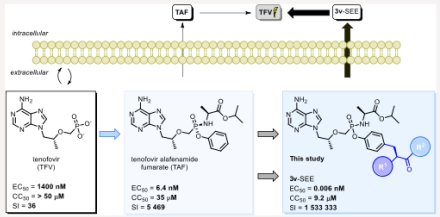
Cite This: J. Med. Chem. 2021, 64, 16425−16449

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

Abstract ➔

ABSTRACT: This study describes the discovery of novel prodrugs bearing tyrosine derivatives instead of the phenol moiety present in FDA-approved tenofovir alafenamide fumarate (TAF). The synthesis was optimized to afford diastereomeric mixtures of novel prodrugs in one pot (yields up to 86%), and the epimers were resolved using a chiral HPLC column into fast-eluting and slow-eluting epimers. In human lymphocytes, the most efficient tyrosine-based prodrug reached a single-digit picomolar EC_{50} value against HIV-1 and nearly 300-fold higher selectivity index (SI) compared to TAF. In human hepatocytes, the most efficient prodrugs exhibited subnanomolar EC_{50} values for HBV and up to 26-fold higher SI compared to TAF. Metabolic studies demonstrated markedly higher cellular uptake of the prodrugs and substantially higher levels of released tenofovir inside the cells compared to TAF. These promising results provide a strong foundation for further evaluation of the reported prodrugs and their potential utility in the development of highly potent antivirals.



Introduction ➔

INTRODUCTION

Both nucleoside phosphates and nucleoside phosphonates are deprotonated at physiological pH, and their ionic character significantly reduces their ability to penetrate through biomembranes. Many types of prodrugs have been developed to address this issue (for comprehensive reviews, see refs 1−4). Prodrug moieties mask the ionic character of the parent molecule and substantially enhance its delivery into the target cell. Once inside the cell, the masking moiety is cleaved off, thus releasing the parent active compound (Figure 1).

The prodrug approach proved to be extremely useful as it significantly enhanced the antiviral activity of the parent nucleotide analogues. For instance, in a cell-based assay, tenofovir (TFV, used in the treatment of retroviral infections[5]) exhibits single digit micromolar potency against human immunodeficiency virus (HIV-1), while the first generation prodrug that utilizes carbonate esters, tenofovir disoproxil fumarate (TDF), exhibits 100-fold higher potency and the second generation prodrug that utilizes the amidate approach, tenofovir alafenamide fumarate (TAF), exhibits 1000-fold higher potency (Figure 2).[6,7]

The efforts to develop efficient phosphate prodrugs started with the application of simple alkyl groups but evolved into the current design, so-called ProTides, a subclass of the phosphoramidate/phosphonamidate prodrugs.[8] The ProTides were initially developed by McGuigan's group in the early 1990s to deliver nucleoside monophosphates,[9] and they are utilized in the FDA-approved drugs, sofosbuvir and remdesivir, and the same approach was also applied to nucleoside phosphonate tenofovir leading to the aforementioned TAF.[10−12]

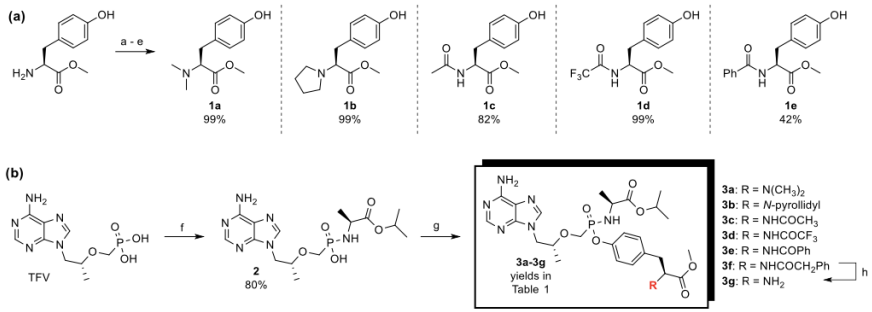
The clinical dose of TDF to achieve the desired efficacy was 300 mg daily due to its low plasma stability.[13] However, this led to high levels of TFV circulating in plasma, which has now been associated with nephrotoxicity and bone toxicity.[7,14] TFV toxicity is promoted by the imbalance between its rapid uptake from plasma (mediated by organic anion transporters (OAT) 1 and 3) and slow efflux into urine (mediated by multidrug resistance protein type 4 (MRP4)), which leads to TFV accumulation in proximal-tubule cells and thus to nephrotoxicity.[15] The mechanism of bone toxicity (reduction of mineral density) is not fully clarified yet, however; no direct effect of TFV on osteoblasts was observed *in vitro*.[16]

The above-mentioned drawbacks of TDF were substantially reduced with TAF, the ProTide of tenofovir, which was more

Received: August 20, 2021
Published: October 29, 2021

Crucial dates

Scheme 1. Synthesis of the First Series of Prodrugs 3[a]



[a]Reagents and conditions: (a) for **1a**: CH_2O, Na_2SO_4, AcOH, $NaBH_3CN$, MeOH, 25 °C, 1 h; (b) for **1b**: 1,4-dibromobutane, $NaHCO_3$, toluene, 115 °C, 3 h; (c) for **1c**: Ac_2O, Na_2CO_3, acetone/H_2O, 25 °C, 30 min; (d) for **1d**: $(CF_3CO)_2O$, pyridine, DCM, 25 °C, 5 min; (e) for **1e**: PhCOOH, HATU, DIPEA, DMF, 25 °C, 1 h; (f) (*S*)-tyrosine methyl ester, EDC, Et_3N, H_2O, 40 °C, 16 h; (g) compound **2**, the corresponding (*S*)-tyrosine derivative **1a**−**1f**, Et_3N, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h; (h) H_2, Pd/C, phosphate buffer (pH 7.4), 25 °C, 1 h.

evidence of the proposed cyclic intermediates in the ProTide activation until recently when Procházková et al.[22] were able to confirm such cyclic species using NMR spectroscopy and mass spectrometry coupled with infrared spectroscopy, supporting the proposed mechanism.

During the ProTide activation, an alcohol, amino acid, and aromatic compound are released. The influence of the amino acid ester was studied by McGuigan et al.[23] Their work indicated that more lipophilic ester may increase the antiviral activity via higher cellular uptake, however, lipophilicity was not the sole determinant of the biological activity. The substrate activity of the compounds toward esterases also played an important role. In particular, *t*-butyl ester was hydrolyzed significantly slower compared to methyl, ethyl, or isopropyl esters. As another example, phenyl ester was cleaved faster compared to more lipophilic alkylphenyl analogues.[23]

The role of the amino acid was also studied. It has been shown that cathepsin A was not able to hydrolyze bulkier amino acids[21] and that (*S*)-alanine was the most suitable option.[21,24] Regarding the aryl moiety, the effects of its substitution were studied on phosphoramidate prodrugs derived from the antiretroviral drug stavudine (d4T) by Siddiqui et al.[25] While mainly electron-withdrawing groups increased the activity of the drug, the toxicity was higher compared to that of the original ProTide that releases phenol.[25] This is one possible reason why phenol is found in the approved amidate prodrugs sofosbuvir and remdesivir. Nevertheless, the release of phenol has been recognized as potentially problematic due to its apparent toxicity.[26,27]

In this study, we focused on replacing the phenol moiety with tyrosine, a natural amino acid, or its derivatives, in order to increase the selectivity index (SI), *i.e.*, toxicity/potency ratio, of the prodrugs. Strikingly, only two tyrosine derivatives of ProTides are known in the literature as a part of an effort to increase the aqueous solubility of d4T (stavudine) by Siddiqui et al.[26] Substitution of phenol for tyrosine methyl ester indeed increased aqueous solubility 100-fold; however, it also decreased the lipophilicity (most likely due to protonation of the free amino group at physiological pH).[26] As a consequence, the potency of d4T was reduced dramatically. The same compound protected with *t*-butyl carbamate (BOC) on the nitrogen exerted the lowest toxicity in the study and partially recovered the activity, which was approximately 10-fold lower than that of the original prodrug bearing phenol.[26] To date, no subsequent study of phenol replacement with tyrosine derivatives has been reported.

This study explores the influence of phenol replacement with tyrosine derivatives on tenofovir with respect to potency, toxicity, and ADME profile. Variations in the amide part of ProTides along with the aryl part would generate a vast number of combinations, which would exceed the scope of this study. Previous studies have shown that (*S*)-alanine isopropyl ester is efficiently cleaved by cathepsin A and is constant between sofosbuvir, remdesivir, and TAF.[21,24] For these reasons, we kept (*S*)-alanine isopropyl ester as the invariable amide part of the prodrug throughout the whole study and only modified the aryl part of the prodrug with various tyrosine derivatives.

■ RESULTS ← Results section

At first, the final prodrugs were prepared sequentially to reduce complexity and potential formation of possible side products (Scheme 1b). However, it was certainly desirable to synthesize prodrugs in a one-pot manner, and during the study the one-pot synthesis was optimized which allowed us to obtain the desired prodrugs in up to 86% yield.

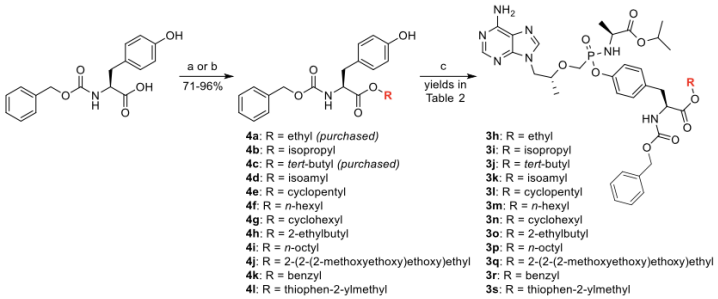
The first series of target prodrugs consisted of (*S*)-tyrosine methyl esters modified on the N-terminus (Scheme 1). Compounds **1a**−**1e** (Scheme 1a) were prepared from (*S*)-tyrosine methyl ester using standard procedures: compound **1a** was obtained via double reductive amination with formaldehyde, compound **1b** was prepared via double nucleophilic displacement of 1,4-dibromobutane, compounds **1c** and **1d** were obtained by the treatment with acetic anhydride and trifluoroacetic anhydride, respectively, under basic conditions, and compound **1e** was obtained via amide coupling with HATU. Compound **1f** was available commercially.

Compound **2** was prepared starting from tenofovir (TFV) according to previously reported procedure with EDC (Scheme 1b).[28] The introduction of the modified tyrosine

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425−16449

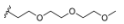
Several pages like this…

Scheme 2. Synthesis of Final Prodrugs in the Second Series[a]



[a]Reagents and conditions: (a) for **4b**: $SOCl_2$, *i*PrOH, 25 °C, 72 h, 79%; (b) for **4d**–**4l**: ROH, PPh_3, DIAD, THF, 0–25 °C, 30 min; (c) compound **2** (Scheme 1), Et_3N or DIPEA, PPh_3, 2,2′-dipyridyl disulfide, pyridine, 65 °C, 16 h.

Table 2. Second Series of Prodrugs, Their Activity Against HIV-1, and Cytotoxicity

Prodrug	R	Yield[a] [%]	EC_{50} [μM]			CC_{50} [μM]		
			FEE	mix[b]	SEE	FEE	mix[b]	SEE
TAF	--	--	--	--	0.0064[c]	--	--	35[c]
3h		32	0.02	0.017	0.0033	13	11	11
3i		24	0.027	0.0016	**0.0006**	13	9.4	10
3j		23		0.013			6.6	
3k		19	0.011	0.0004	**0.00056**	24	8.4	8.0
3l		37	0.016	0.0009	**0.00007**	17	8.3	10
3m		37		0.0018			8.4	
3n		53	0.023	n/a	0.016	17	n/a	13
3o		56	0.021	n/a	0.0084	14	n/a	9.1
3p		43	0.036	0.038	0.050	38	27	38
3q		32		0.47			>50	
3r		40		0.009			10	
3s		10		0.0034			8.2	

[a]Isolated yield of the epimeric mixture. [b]Data for the mixture of epimers. [c]Data for S_P-epimer (TAF).

[a]Isolated yield of the epimeric mixture. [b]Data for the mixture of epimers. [c]Data for S_P-epimer (TAF).

final prodrugs **3h**–**3s** (Scheme 2, Table 2) in 10–56% yields. Again, chiral HPLC was employed in order to separate the fast-eluting epimer (FEE) and the slow-eluting epimer (SEE) of the selected prodrugs (Table 2).

In order to prepare target prodrugs on larger scales, the one-pot synthesis was optimized using the preparation of prodrug **3l** as the model reaction (Scheme 3; for details, see the Supporting Information). The best yield of **3l** (86%) was obtained when TFV (1.0 equiv) in pyridine was stirred with (*S*)-alanine isopropyl ester hydrochloride (1.0 equiv), (*S*)-tyrosine derivative (1.8 equiv), and DIPEA (10 equiv) at 65 °C, and subsequently, a mixture of PPh_3 (4.0 equiv) and 2,2′-dipyridyl disulfide (4.0 equiv) in pyridine was added. The yield of **3l** prepared by the one-pot procedure (86%) was superior to that reached by the sequential method (37%).

No obvious correlation between potency and lipophilicity and/or bulkiness of the (*S*)-tyrosine ester group was observed (Table 2): ethyl ester **3h** and *t*-butyl ester **3j** exhibited 1 order

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425–16449

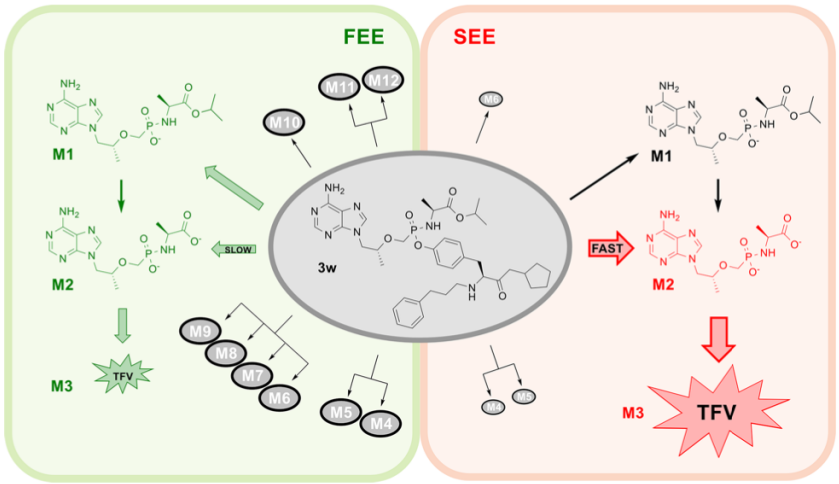


Figure 12. Intracellular metabolization of prodrug **3w** demonstrating the sharp differences in the metabolism of FEE and SEE.

Table 5. Observed Metabolites of FEE and SEE of Prodrug 3w

metabolite	most possible transformation	change	exact mass	most possible formula	FEE/SEE abundance
M1	tyrosine cleavage	$-C_{24}H_{29}NO$	400.162	$C_{15}H_{25}N_6O_5P$	5-fold ↑ in FEE
M2 (**2**)	*i*Pr ester cleavage → cyclization → hydrolysis	$-C_{27}H_{35}NO$	358.116	$C_{12}H_{19}N_6O_5P$	11-fold ↑ in SEE
M3 (TFV)	(*S*)-alanine cleavage	$-C_{30}H_{40}N_2O_2$	287.078	$C_9H_{14}N_5O_4P$	6-fold ↑ in SEE
M4	reduction	$+H_2$	749.403	$C_{39}H_{56}N_7O_8P$	13.5-fold ↑ in FEE
M5	reduction	$+H_2$	749.403	$C_{39}H_{56}N_7O_8P$	1.5-fold ↑ in FEE
M6	desaturation, oxidation	$-H_2+O$	761.367	$C_{39}H_{52}N_7O_7P$	7-fold ↑ in FEE
M7	desaturation, oxidation	$-H_2+O$	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M8	desaturation, oxidation	$-H_2+O$	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M9	desaturation, oxidation	$-H_2+O$	761.367	$C_{39}H_{52}N_7O_7P$	only in FEE
M10	oxidative deamination	$-NH+O$	748.371	$C_{39}H_{53}N_6O_7P$	only in FEE
M11	oxidation	$+O$	763.382	$C_{39}H_{54}N_7O_7P$	only in FEE
M12	oxidation	$+O$	763.382	$C_{39}H_{54}N_7O_7P$	only in FEE

mechanism of ProTide activation. Moreover, **3w**-FEE metabolism resulted in a considerably higher number of observed metabolites (Figure 12, Table 5). This could be explained by the evidently slower cleavage of **3w**-FEE to TFV which leaves higher levels of intact prodrug available for sideways metabolic transformations by other enzymes as opposed to **3w**-SEE, which is rapidly cleaved to the parent TFV thus reducing competition with unproductive metabolism pathways.

We also verified the eligibility of prepared prodrugs for hepatocytes. The prodrugs were evaluated for their anti-HBV activity in HepG2-hNTCP cells (Table 6). Thirteen prodrugs (either as separated epimers or as mixtures) exhibited higher efficacy compared to TAF, and in most cases the prodrugs did not exert any cytotoxicity. The most promising anti-HBV compounds with subnanomolar EC_{50} values were **3k** (EC_{50} = 0.20 nM) and **3f** (EC_{50} = 0.60 nM) as epimeric mixtures and **3d**-SEE (EC_{50} = 0.27 nM) as the single epimer. Compounds **3k** and **3d**-SEE exhibited more than 26-fold and 20-fold higher SI, respectively, compared to TAF.

■ CONCLUSIONS AND DISCUSSION

Conclusions…

Based on the classic ProTide technology, modified prodrug moieties were developed replacing phenol with (*S*)-tyrosine, in order to increase potency and/or selectivity index (SI, *i.e.*, toxicity/potency ratio). Tenofovir was selected as a convenient parent compound for this study, and the anti-HIV-1 activity of the novel prodrugs was compared to the ProTide prodrug of tenofovir, *i.e.*, tenofovir alafenamide fumarate (TAF). The synthesis was performed sequentially at first; however, it was considered highly desirable to synthesize the desired prodrugs in a one-pot manner. Hence, a one-pot reaction methodology was developed reaching up to 86% yield which was considerably higher than the yield of sequential synthesis (31% in average over the two steps, 59% the best yield).

During the synthesis, a new chiral center was formed on the phosphorus atom. The two created epimers were resolved using a chiral HPLC column, thus affording a fast-eluting epimer (FEE) and a slow-eluting epimer (SEE). It has been suggested (based on CD spectra, HPLC data, prodrug metabolism) that the more potent SEE derivatives have the

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425−16449

Table 6. Anti-HBV Activity and Cytotoxicity of Studied Prodrugs in HepG2-hNTCP Cells

	EC_{50} [μM]			CC_{50} [μM]		
prodrug	FEE	mix[a]	SEE	FEE	mix[a]	SEE
TAF[b]			0.0053			>50
3c	0.0072		0.0032	>50		>50
3d	0.0016		0.00027	>50		>50
3f		0.0006			15	
3h		0.0036			>50	
3i		0.0042			>50	
3k		0.0002			>50	
3l		0.0007			>50	
3m		0.0014			>50	
3v	0.0034		0.0028	>50		>50
3x	0.0014		0.0044	>50		>50
3y	0.0040		0.0190	>50		>50
11		0.0013			46	
15		0.0012			>50	

[a]Data for the mixture of epimers. [b]Data for S_p-epimer (TAF).

same phosphorus stereochemistry as TAF (*i.e.*, S_p-epimer), but numerous attempts (X-ray, cryo-EM) to determine the absolute configuration of the epimers have failed so far.

The first series of prodrugs was modified on the N-terminus of tyrosine, while the second series was modified on the C-terminus of tyrosine. The anti-HIV-1 data showed the SEEs to be the more potent epimers. The promising prodrug **3l**-SEE was discovered with a double-digit picomolar EC_{50} value and 26-fold higher SI compared to TAF. Prodrug **3l**-SEE was therefore thoroughly evaluated. It was observed that the chiral center on tyrosine played an important role as the analogous prodrug to **3l** bearing (*R*)-tyrosine, *i.e.*, compound **3t**, exhibited 1 order of magnitude lower efficacy. The water-solubility of **3l**-SEE was increased 2-fold and 4-fold *via* the formation of fumarate and hydrochloride salts, respectively. Prodrug **3l**-SEE was further evaluated with respect to its chemical and metabolic stability. While exhibiting similar chemical stability profiles as TAF, **3l**-SEE proved to be slightly more stable within the pH range of 1.5−10. The plasma stability of **3l**-SEE was substantially higher than that of TAF. Nevertheless, prodrug **3l**-SEE degraded almost immediately in human hepatic microsomes. The major metabolite was identified as the compound lacking the cyclopentyl moiety on the (*S*)-tyrosine carboxyl group. Therefore, the ester function was replaced by an isosteric amide or ketone moieties to address this issue. While the substitution of the ester with amide (**3u**) led to a dramatic drop of efficacy, the substitution of the ester with ketone (**3v**) resulted in a single-digit picomolar EC_{50} and nearly 300-fold increase of SI compared to TAF. Prodrug **3v**-SEE reached EC_{50} = 6 pM and a SI over 1 500 000 and thus represents the most potent anti-HIV-1 ProTide ever reported.

The most promising prodrugs in the whole study (**3v**, **3w**) also proved to be significantly more stable in plasma compared to TAF. More importantly, the microsomal stability was increased; however, it remained considerably lower compared to TAF. Further studies aimed at increasing the microsomal stability of these prodrugs are in progress.

The reason for the outstanding potency of the prodrugs was also investigated. The tyrosine residues which are released during the prodrug activation exhibited no activity against HIV-1 and therefore could not explain any increase in antiviral potency. Faster metabolization by cathepsin A was also excluded as a potential reason, since the novel prodrugs proved to be comparable substrates for cathepsin A as TAF. The reason for the exceptional potency appears to be a very high cellular uptake of the prodrugs and their rapid cleavage resulting in high levels of free tenofovir in the target cells. Subsequent metabolic studies further revealed substantial differences in the metabolism of FEE and SEE.

The prepared prodrugs were also studied against HBV in human hepatocytes. Thirteen prodrugs demonstrated higher potency compared to TAF while showing no cytotoxicity. The most efficient prodrug in the study against HBV proved to be **3k**, exhibiting over 26-fold higher SI compared to TAF.

These promising results support further development of next generation (*S*)-tyrosine-based prodrugs with the aim to increase microsomal stability while retaining the extraordinary potency.

■ EXPERIMENTAL SECTION

← The details

Chemistry. *General Information.* All solvents and reagents were purchased from commercial suppliers and were not further purified. TAF was donated by Gilead Sciences, Inc., Foster City, CA, USA. Reactions were monitored using thin-layer chromatography (TLC) on silica gel 60 F254 plates (Merck KGaA, Germany) and/or ultrahigh performance liquid chromatography with mass spectrometry (UPLC-MS Acquity Waters, USA, H-Class Core System with Waters Acquity UPLC BEH C_{18} 1.7 μm, 2.1 × 100 mm column, Waters Acquity UPLC PDA detector, and mass spectrometer Waters SQD2, linear gradient elution with 0−100% MeCN in water with 0.1% HCOOH). Column and flash chromatography (ISCO Teledyne, USA) were performed on 60 Å silica gel (Acros Organics, Belgium). Solvents were evaporated using rotary evaporator at 40−70 °C/2 mbar.

NMR spectra were measured using a Bruker Avance III HD 400 MHz instrument equipped with a Prodigy cryoprobe operating at 9.39 T or a Bruker Avance III HD 500 MHz instrument equipped with a cryoprobe operating at 11.74 T in DMSO-d_6 solution. Using the 400 MHz spectrometer, the ^{1}H, ^{13}C, ^{19}F, and ^{31}P spectra were acquired at 400, 100, 377, and 162 MHz, respectively. Using the 500 MHz spectrometer, the ^{1}H, ^{13}C, ^{19}F, and ^{31}P spectra were acquired at 500, 125, 470, and 202 MHz, respectively. Two-dimensional spectra ^{1}H^{1}H COSY, ^{1}H^{13}C HSQC, ^{1}H^{13}C HMBC were acquired for assignment purposes. Chemical shifts (δ) are listed in ppm, and coupling constants (J) in are given in Hz. NMR multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of doublets of doublets of doublets (dddd), doublet of triplets (dt), doublet of quartets (dq), doublet of pentets (dp), triplet of doublets (td), and multiplet (m). All spectra were referenced to the residual signal of DMSO (2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C). For numbering of final compounds, see Figure 13. High-resolution mass spectra were obtained using a LTQ Orbitrap XL instrument (Thermo Fisher Scientific, USA) for ESI ionization.

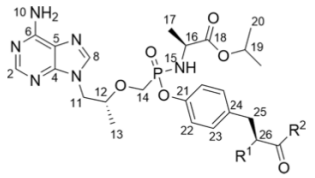


Figure 13. Template used for numbering of final prodrugs used for assignments of NMR spectra. Specific numbering for each prodrug is given in the Supporting Information.

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425−16449

Several pages like this…

7.40 (m, 3H), 7.13−7.05 (m, 2H), 6.69−6.61 (m, 2H), 4.58 (ddd, J = 9.9, 7.8, 5.4 Hz, 1H), 3.63 (s, 3H), 3.09−2.93 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.39, 166.50, 155.96, 133.74, 131.52, 130.04, 128.32, 127.72, 127.43, 115.08, 54.73, 51.92, 35.55. **HRMS** (ESI) m/z [M + H]$^+$ calcd for $C_{17}H_{18}O_4N$ 300.12303, found 300.12298. $[\alpha]^{25}_D$ = +6.0 (c 0.252 g/100 mL, $CHCl_3$/MeOH 1/1).

*P-((((R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)-N-((S)-1-isopropoxy-1-oxopropan-2-yl)phosphonamidic Acid (**2**).* Tenofovir (500 mg, 1.74 mmol), (*S*)-alanine isopropyl ester hydrochloride (1.46 g, 8.71 mmol), and DIPEA (1.52 mL, 8.71 mmol) were mixed in H_2O (20 mL). EDC (1.35 g, 8.71 mmol) was added, and the mixture was stirred at 40 °C for 24 h, concentrated, and separated using C_{18}-reversed phase flash chromatography (linear gradient elution 0−100% MeOH in water) to afford **2** (558 mg, 80%) as a white solid. **^{1}H NMR** (500 MHz, D_2O) δ 8.22 (s, 2H, *H2, H8*), 4.69 (sept, J = 6.3 Hz, 1H, *H19*), 4.36 (dd, J = 14.8, 3.0 Hz, 1H, *H11a*), 4.20 (dd, J = 14.8, 7.9 Hz, 1H, *H11b*), 3.95−3.88 (m, 1H, *H12*), 3.62−3.57 (m, 2H, *H14a, H16*), 3.31 (dd, J = 12.6, 9.7 Hz, 1H, *H14b*), 1.21−1.05 (m, 12H, *H13, H17, H20*). **^{13}C NMR** (126 MHz, D_2O) δ 177.13 (d, J = 3.3 Hz, *C18*), 156.12 (*C6*), 152.91 (*C2*), 149.85 (*C4*), 144.11 (*C8*), 118.90 (*C5*), 76.34 (d, J = 12.4 Hz, *C12*), 70.41 (*C19*), 67.19−66.01 (m, *C14*), 50.66 (*C16*), 49.02 (*C11*), 21.30 and 21.28 (*C20*), 21.01 (d, J = 5.4 Hz, *C17*), 16.37 (*C13*). **^{31}P NMR** (162 MHz, D_2O) δ 19.69. **HRMS** (ESI) m/z [M + H]$^+$ calcd for $C_{15}H_{26}O_5N_6P$ 401.16968, found 401.17043.

*Methyl (2S)-3-(4-((((((R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(dimethylamino)propanoate (**3a**).* Following standard procedure C, **2** (300 mg, 0.75 mmol), **1a** (301 mg, 1.35 mmol), DIPEA (1.05 mL, 6.00 mmol), PPh_3 (787 mg, 3.00 mmol), and 2,2′-dipyridyl disulfide (661 mg, 3.00 mmol) were stirred in pyridine (7 mL) to afford **3a** (94 mg, 21%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.14 (s, 1H, *H2*), 8.11 and 8.11 (s, 1H, *H8*), 7.22 and 7.21 (bs, 1H, *H10*), 7.18−7.09 (m, 2H, *H23*), 7.03−6.93 (m, 2H, *H22*), 5.62 and 5.49 (dd, J = 12.0, 10.4 and 12.3, 9.9 Hz, 1H, *H15*), 4.90−4.78 (m, 1H, *H19*), 4.32−4.10 (m, 2H, *H11*), 4.01−3.71 (m, 4H, *H12, H14, H16*), 3.54 (s, 3H, *H29*), 3.44−3.38 (m, 1H, *H26*), 2.93−2.77 (m, 2H, *H25*), 2.25 (s, 6H, *H27*), 1.18−1.00 (m, 12H, *H13, H17, H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.05 and 172.93 (d, J = 4.0 and 4.0 Hz, *C18*), 171.13 (*C28*), 156.01 and 155.99 (*C6*), 152.45 (*C2*), 149.87 and 149.83 (*C4*), 148.70−148.49 (m, *C21*), 141.49 and 141.44 (*C8*), 134.38 and 134.30 (*C24*), 130.00 (*C23*), 120.44 and 120.24 (d, J = 4.3 and 4.4 Hz, *C22*), 118.42 and 118.36 (*C5*), 75.75−75.41 (m, *C12*), 68.23 (*C26*), 67.94 (*C19*), 64.21 and 64.14 (d, J = 155.1 and 154.8 Hz), 50.75 (*C29*), 49.11 and 49.03 (*C16*), 46.84 and 46.74 (*C11*), 41.27 (*C27*), 34.21 (*C25*), 21.48−21.39 (m, *C20*), 20.28 and 19.91 (d, J = 5.1 and 5.5 Hz, *C17*), 16.76 and 16.65 (*C13*). **^{31}P NMR** (DMSO-d_6, mixture of epimers) δ 25.53 and 24.73. **HRMS** (ESI) m/z [M + H]$^+$ calcd for $C_{27}H_{41}O_7N_7P$ 606.27996, found 606.27982.

*Methyl (2S)-3-(4-((((((R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(pyrrolidin-1-yl)propanoate (**3b**).* Following standard procedure C, **2** (188 mg, 0.47 mmol), **1b** (209 mg, 0.84 mmol), DIPEA (655 μL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and 2,2′-dipyridyl disulfide (414 mg, 1.88 mmol) were stirred in pyridine (5 mL) to afford **3b** (160 mg, 54%) as a white solid. **^{1}H NMR** (401 MHz, DMSO-d_6, mixture of epimers) δ 8.14 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.22 and 7.20 (bs, 2H, *H10*), 7.17−7.06 (m, 2H, *H23*), 7.03−6.92 (m, 2H, *H22*), 5.62 and 5.48 (dd, J = 12.0, 10.4 and 12.4, 10.0 Hz, 1H, *H15*), 4.89−4.78 (m, 1H, *C19*), 4.31−4.09 (m, 2H, *H11*), 3.99−3.72 (m, 4H, *H12, H14, H16*), 3.50 (s, 3H, *H30*), 3.47−3.44 (m, 1H, *H26*), 2.93−2.87 (m, 2H, *H25*), 2.66−2.55 (m, 4H, *H27*), 1.68−1.62 (m, 4H, *H28*), 1.18−1.02 (m, 12H, *H13, H17, H20*). **^{13}C NMR** (101 MHz, DMSO-d_6, mixture of epimers) δ 173.03 and 172.90 (d, J = 4.1 and 3.9 Hz, *C18*), 171.69 (*C28*), 155.99 and 155.97 (*C6*), 152.43 (*C2*), 149.85 and 149.81 (*C4*), 148.84−148.54 (m, *C21*), 141.46 and 141.41 (*C8*), 134.14 and 134.05 (*C24*), 129.94 (*C23*), 120.44 and 120.20 (d, J = 4.3 and 4.5 Hz, *C22*), 118.40 and 118.35 (*C5*), 75.75−75.31 (m, *C12*), 67.91 (*C19*), 66.34 (*C26*), 64.18 and 64.12 (d, J = 154.4 and 155.1 Hz, *C14*), 50.86 (*C30*), 49.28 (*C27*), 49.09 and 49.00 (*C16*), 46.82 and 46.71 (*C11*), 35.83 (*C25*), 23.11 (*C28*), 21.48−21.36 (m, *C20*), 20.24 and 19.89 (d, J = 5.2 and 5.5 Hz, *C17*), 16.76 and 16.62 (*C13*). **^{31}P NMR** (162 MHz, DMSO-d_6, mixture of epimers) δ 25.52 and 24.67. **HRMS** (ESI) m/z [M + H]$^+$ calcd for $C_{29}H_{43}O_7N_7P$ 632.29561, found 632.29538.

*Methyl (2S)-2-Acetamido-3-(4-((((((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate (**3c**).* Following standard procedure C, **2** (120 mg, 0.30 mmol), **1c** (85 mg, 0.36 mmol), Et_3N (335 μL, 2.40 mmol), PPh_3 (315 mg, 1.20 mmol), and 2,2′-dipyridyl disulfide (264 mg, 1.20 mmol) were stirred in pyridine (5 mL) to afford **3c** (55 mg, 30%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 8.35 and 8.33 (bs, 1H, *H27*), 8.13 and 8.13 (s, 1H, *H2*), 8.11 and 8.10 (s, 1H, *H8*), 7.26−7.12 (m, 4H, *H10, H23*), 7.04−6.95 (m, 2H, *H22*), 5.68−5.60 and 5.55−5.46 (m, 1H, *H15*), 4.90−4.78 (m, 1H, *H19*), 4.45−4.37 (m, 1H, *H26*), 4.31−4.10 (m, 2H, *H11*), 4.00−3.89 (m, 1H, *H12*), 3.87−3.71 (m, 3H, *H14, H16*), 3.58 and 3.56 (s, 3H, *H31*), 3.00−2.79 (m, 2H, *H25*), 1.78 and 1.78 (s, 3H, *H29*), 1.19−1.01 (m, 12H, *H13, H17, H20*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.07 and 172.93 (d, J = 4.1 and 4.0 Hz, *C18*), 172.23 (*C30*), 169.36 (*C28*), 156.00 and 155.98 (*C6*), 152.46 and 152.44 (*C2*), 149.85 and 149.81 (*C4*), 149.09−148.72 (m, *C21*), 141.47 and 141.43 (*C8*), 133.35 and 133.25 (*C24*), 130.09 (*C23*), 120.53 and 120.25 (d, J = 4.1 and 4.6 Hz, *C22*), 118.38 and 118.34 (*C5*), 75.82−75.31 (m, *C12*), 67.94 (*C19*), 64.21 and 64.13 (d, J = 154.8 and 155.2 Hz, *C14*), 53.64 (*C26*), 51.85 (*C31*), 49.09 and 49.00 (*C16*), 46.79 and 46.68 (*C11*), 36.00 (*C25*), 22.25 (*C29*), 21.48−21.41 (m, *C20*), 20.28 and 19.92 (d, J = 5.2 and 5.4 Hz, *C17*), 16.82 and 16.67 (*C13*). **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.90 and 22.03. **HRMS** (ESI) m/z [M + Na]$^+$ calcd for $C_{27}H_{38}O_8N_7NaP$ 642.24117, found 642.24054.

*Methyl (2S)-3-(4-((((((R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-(2,2,2-trifluoroacetamido)propanoate (**3d**).* Following standard procedure C, **2** (150 mg, 0.37 mmol), **1d** (216 mg, 0.74 mmol), Et_3N (413 μL, 2.96 mmol), PPh_3 (388 mg, 1.48 mmol), and 2,2′-dipyridyl disulfide (326 mg, 1.48 mmol) were stirred in pyridine (6 mL) to afford **3d** (13 mg, 5%) as an off-white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, mixture of epimers) δ 9.90 (bs, 1H, *H27*), 8.13 and 8.13 (s, 1H, *H2*), 8.10 and 8.09 (s, 1H, *H8*), 7.24−7.16 (m, 4H, *H10, H23*), 7.05−6.99 (m, 2H, *H22*), 5.68−5.60 (m) and 5.48 (dd, J = 12.4, 10.0 Hz, 1H, *H15*), 4.88−4.78 (m, 1H, *H19*), 4.63−4.55 (m, 1H, *H26*), 4.30−4.11 (m, 2H, *H11*), 3.99−3.89 (m, 1H, *H12*), 3.88−3.72 (m, 3H, *H14, H16*), 3.67 and 3.67 (s, 3H, *H31*), 3.19−3.13 and 3.01−2.95 (m, 1H, *H25*), 1.18−1.02 (m, 12H, *H13, H17, H20*). **^{13}C NMR** (126 MHz, DMSO-d_6, mixture of epimers) δ 173.02 and 172.86 (d, J = 4.3 and 4.0 Hz, *C18*), 170.38 (*C30*), 156.32 and 156.30 (q, J = 36.6 and 36.2 Hz, *C28*), 155.97 and 155.96 (*C6*), 152.43 and 152.41 (*C2*), 149.84 and 149.80 (*C4*), 149.20−148.77 (m, *C21*), 141.44 and 141.40 (*C8*), 132.88 and 132.73 (*C24*), 130.09 (*C23*), 120.61 and 120.23 (d, J = 4.2 and 4.6 Hz, *C22*), 118.37, 118.33 (*C5*), 115.65 (q, J = 288.0 Hz, *C29*), 75.78−75.39 (m, *C12*), 67.89 (*C19*), 64.21 and 64.11 (d, J = 155.0 and 154.9 Hz, *C14*), 53.91 (*C26*), 52.42 (*C31*), 49.06 and 48.97 (*C16*), 46.75 and 46.67 (*C11*), 34.80 (*C25*), 21.46−21.32 (m, *C20*), 20.18 and 19.83 (d, J = 5.1 and 5.2 Hz, *C17*), 16.77 and 16.63 (*C13*). **^{19}F NMR** (470 MHz, DMSO-d_6, mixture of epimers) δ −74.26 and −74.28. **^{31}P NMR** (202 MHz, DMSO-d_6, mixture of epimers) δ 22.93 and 22.02. **HRMS** (ESI) m/z [M + Na]$^+$ calcd for $C_{27}H_{35}O_8N_7F_3NaP$ 696.21290, found 696.21216.

*Methyl (2S)-3-(4-((((((R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)-2-benzamidopropanoate (**3e**).* Following standard procedure C, **2** (188 mg, 0.47 mmol), **1e** (251 mg, 0.84 mmol), DIPEA (655 μL, 3.76 mmol), PPh_3 (493 mg, 1.88 mmol), and 2,2′-dipyridyl disulfide (414 mg, 1.88 mmol) were stirred in

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425−16449

guard, 40 °C, flow rate 1 mL/min, modified gradient 5−100% of MeCN in H_2O), this vial was used to measure an 8-point calibration curve (based on the UV absorption at 254 nm) ranging from 1.35 to 150 μM via different injection volumes.

From the stock solution of the studied compound (0.5 mM in DMSO), 50 μL was pipetted in a vial containing 950 μL of appropriate buffer affording a 25 μM concentration. Each sample was incubated at a given temperature (4 °C, 22 °C, or 37 °C) for 24 h while analyzing the sample every 3 h. Each experiment was conducted in triplicate at three temperatures and five different pHs, thus resulting in 45 experiments for each compound.

Plasma and Microsomal Stability Assay. To determine plasma stability of studied prodrugs, 5 μM of these were incubated with human pooled plasma from 50 donors (Biowest) for 120 and 240 min at 37 °C. The reactions were terminated by adding four volumes of ice-cold methanol, and the samples were then mixed vigorously and left at −20 °C for 1 h. After that, the samples were centrifuged and the supernatant was analyzed by means of an ECHO-MS System (Sciex). Zero time points were prepared by adding ice-cold methanol to the compound prior the addition of the plasma.

Microsomal stability assay was performed using the 0.5 mg/mL human pooled microsomal preparation (Thermo Scientific) and 5 μM compounds in 90 mM TRIS-Cl buffer pH 7.4 containing 2 mM NADPH and 2 mM $MgCl_2$ for 5, 10, and 30 min at 37 °C. The reactions were terminated by the addition of four volumes of ice-cold methanol and further processed using the same procedures as described above.

Enzymatic Hydrolysis. Purified cathepsin A (2.2 μg/mL) was incubated with 30 μM of the studied prodrugs and TAF at 37 °C in a reaction buffer containing 25 mM 2-(*N*-morpholinoethanesulfonic) acid (MES), 100 mM NaCl, 1 mM DDT, and 0.1% NP40 (pH 6.5) in 40 μL aliquots. At set time points, the reaction was stopped by adding 40 μL of ice-cold 100% methanol. The samples were incubated at −20 °C for 30 min and centrifuged at 13 000*g* for 30 min at 4 °C to remove denatured proteins. The supernatant aliquots (10 μL) were injected onto a C18 reverse phase Acquity UPLC HSS T3 column (1.8 μm; 2.1 × 150 mm; Waters, Milford, MA, USA) equilibrated with buffer A (25 mM potassium phosphate, pH 6.0). Linear gradient elution was used (0−90% acetonitrile in 10 min in buffer A) with flow rate 0.25 mL/min.

Intracellular Uptake and Tenofovir Release Assay. CCRF-CEM cells were washed by centrifugation (250*g*, 5 min) in PBS and resuspended in RPMI medium without any additives. The cell suspension was distributed into microtubes in 500 μL aliquots, and 2.5 μL of the 10 mM DMSO solutions of the prodrugs was added to reach a concentration of 50 μM. Samples were incubated for 45 min at 37 °C in a CO_2 incubator using a rotary stirrer. Blank samples without the presence of the cells were also prepared and used for the estimation of the initial concentration of the compounds at time zero and compound recovery. The uptake process was terminated by centrifugation at 900*g* for 5 min, and the medium was stored at −20 °C for further analysis of extracellular concentration of the prodrugs. The cells were washed two times in PBS (900*g*), and the final pellet was stored at −20 °C for subsequent analysis of the intracellular concentration of the prodrugs and their metabolites. Prior to the analysis, aliquots of the medium with or without the cells and the cell pellet were extracted with ice-cold methanol by vigorous shaking and centrifuged at 20 000*g* for 10 min and the supernatants were 10× diluted and subjected to the analysis by ECHO-MS (Sciex). Calibration curves of the prodrugs and (*R*)-PMPA (tenofovir) in 50% methanol were measured alongside the samples.

■ ASSOCIATED CONTENT

More information →

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01444.

Optimization of the one-pot preparation of the prodrugs, formation of **3l**-SEE salts, calibration curves, levels of confidence for EC_{50}'s and CC_{50}'s from Tables 1−3, chiroptical vibrational spectra of **3l**, specific numbering for assignments of NMR signals, copies of spectra of the prepared compounds (PDF)

Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Author

Zlatko Janeba − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic;* orcid.org/0000-0003-4654-679X; Phone: +420 220 183 143; Email: janeba@uochb.cas.cz

the authors ↓

Authors

Filip Kalčic − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic; Department of Organic Chemistry, Faculty of Science, Charles University, 128 43 Prague 2, Czech Republic*

Michala Zgarbová − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Jan Hodek − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Karel Chalupský − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Martin Dračínský − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic;* orcid.org/0000-0002-4495-0070

Alexandra Dvořáková − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Timotej Strmeň − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Jaroslav Šebestík − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Ondřej Baszczyňski − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic; Department of Organic Chemistry, Faculty of Science, Charles University, 128 43 Prague 2, Czech Republic*

Jan Weber − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Helena Mertlíková-Kaiserová − *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c01444

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

← Acknowledgements

This research was funded by the Institute of Organic Chemistry and Biochemistry (RVO 61388963) and by the Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (Project LTAU-

SA18086). This work was supported by computational grants of CESNET (LM2015042) and the CERIT-SC (LM2015085). We would like to thank Dr. Richard Mackman (Gilead Sciences, Inc., Foster City, CA, USA) for his helpful comments and for editing of the manuscript. We are grateful to the MS department at IOCB (HRMS), Lucie Holasová (OR), Lucie Bednárová (CD), and Jiří Rybáček (SFC) for their help with characterization of prepared compounds.

Dedication ➔

DEDICATION

This work is dedicated to the memory of Dana Hocková (1970−2021), a brilliant scientist, an excellent friend, and a long-standing colleague at the Institute of Organic Chemistry and Biochemistry in Prague.

Abbreviations ➔

ABBREVIATIONS USED

AUC, area under curve; d4T, stavudine; FEE, fast-eluting epimer; FTC, emtricitabine; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IC, intracellular content; MRP4, multidrug resistance protein type 4; OAT, organic anion transporters; SEE, slow-eluting epimer; SI, selectivity index, *i.e.* toxicity/potency ratio; $t_{1/2}$, half-time; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; TFVpp, tenofovir diphosphate

References ➔

REFERENCES

(1) Pradere, U.; Garnier-Amblard, E. C.; Coats, S. J.; Amblard, F.; Schinazi, R. F. Synthesis of Nucleoside Phosphate and Phosphonate Prodrugs. *Chem. Rev.* **2014**, *114* (18), 9154−9218.
(2) Heidel, K. M.; Dowd, C. S. Phosphonate Prodrugs: An Overview and Recent Advances. *Future Med. Chem.* **2019**, *11* (13), 1625−1643.
(3) Pertusat, F.; Serpi, M.; Mcguigan, C. Medicinal Chemistry of Nucleoside Phosphonate Prodrugs for Antiviral Therapy. *Antivir. Chem. Chemother.* **2012**, *22* (5), 181−203.
(4) Krise, J. P.; Stella, V. J. Prodrugs of Phosphates, Phosphonates, and Phosphinates. *Adv. Drug Delivery Rev.* **1996**, *19* (2), 287−310.
(5) Naesens, L.; Bischofberger, N.; Augustijns, P.; Annaert, P.; Van den Mooter, G.; Arimilli, M. N.; Kim, C. U.; De Clercq, E. An
lox
in
(6
Sch
Ant
Nu
Ant
Dia
338
(7
Ala
Hu
(8) Mehellou, Y.; Rattan, H. S.; Balzarini, J. The ProTide Prodrug Technology: From the Concept to the Clinic. *J. Med. Chem.* **2018**, *61* (6), 2211−2226.
(9) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. Aryl Phosphate Derivatives
HIV1 in Cell Lines Which Are Re
Antiviral Res. **1992**, *17* (4), 311−321
(10) Thornton, P. J.; Kadri, H.; Mic
Phosphate and Phosphonate Prodrug Clinical Candidates. *J. Med. Chem.* **2016**, *59* (23), 10400−10410.
(11) De Clercq, E.; Li, G. Approved Antiviral Drugs over the Past 50 Years. *Clin. Microbiol. Rev.* **2016**, *29* (3), 695−747.
(12) Eastman, R. T.; Roth, J. S.; Brimacombe, K. R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M. D. Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent. Sci.* **2020**, *6*, 672.
(13) Golla, V. M.; Kurmi, M.; Shaik, K.; Singh, S. Stability Behaviour of Antiretroviral Drugs and Their Combinations. 4: Characterization of Degradation Products of Tenofovir Alafenamide Fumarate and Comparison of Its Degradation and Stability Behaviour with Tenofovir Disoproxil Fumarate. *J. Pharm. Biomed. Anal.* **2016**, *131* (May), 146−155.
(14) Ruane, P. J.; Dejesus, E.; Berger, D.; Markowitz, M.; Bredeek, U. F.; Callebaut, C.; Zhong, L.; Ramanathan, S.; S. Rhee, M.; Fordyce, M. W.; Yale, K. Antiviral Activity, Safety, and Pharmacokinetics/Pharmacodynamics of Tenofovir Alafenamide as 10-Day Monotherapy in HIV-1-Positive Adults. *JAIDS, J. Acquired Immune Defic. Syndr.* **2013**, *63* (4), 449−455.
(15) Ray, A. S.; Cihlar, T.; Robinson, K. L.; Tong, L.; Vela, J. E.; Fuller, M. D.; Wieman, L. M.; Eisenberg, E. J.; Rhodes, G. R. Mechanism of Active Renal Tubular Efflux of Tenofovir. *Antimicrob. Agents Chemother.* **2006**, *50* (10), 3297−3304.
(16) Liu, Y.; Kitrinos, K.; Babusis, D.; Ray, A. S.; Miller, M. D.; Callebaut, C. Lack of Tenofovir Alafenamide (TAF) Effect on Primary Osteoblasts in Vitro at Clinically Relevant Drug Concentrations. In *53rd Interscience Conference on Antimicrobial Agents and Chemotherapy*; 2013; p H-664.
(17) De Clercq, E. Tenofovir Alafenamide (TAF) as the Successor of Tenofovir Disoproxil Fumarate (TDF). *Biochem. Pharmacol.* **2016**, *119*, 1−7.
(18) Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J. Synthesis and Evaluation of Novel Amidate Prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.* **2001**, *11* (8), 1053−1056.
(19) Ray, A. S.; Fordyce, M. W.; Hitchcock, M. J. M. Tenofovir Alafenamide: A Novel Prodrug of Tenofovir for the Treatment of Human Immunodeficiency Virus. *Antiviral Res.* **2016**, *125*, 63−70.
(20) Podany, A. T.; Bares, S. H.; Havens, J.; Dyavar, S. R.; O'Neill, J.; Lee, S.; Fletcher, C. V.; Swindells, S.; Scarsi, K. K. Plasma and Intracellular Pharmacokinetics of Tenofovir in Patients Switched from Tenofovir Disoproxil Fumarate to Tenofovir Alafenamide. *AIDS* **2018**, *32* (6), 761−765.
(21) Birkus, G.; Wang, R.; Liu, X.; Kutty, N.; MacArthur, H.; Cihlar, T.; Gibbs, C.; Swaminathan, S.; Lee, W.; McDermott, M. Cathepsin a Is the Major Hydrolase Catalyzing the Intracellular Hydrolysis of the Antiretroviral Nucleotide Phosphonoamidate Prodrugs GS-7340 and GS-9131. *Antimicrob. Agents Chemother.* **2007**, *51* (2), 543−550.
J.;
Tide
ane.
ner, K.; O'Leary,
rini, J. Synthesis,
Esterase Lability
horamidate De-
Chemother. **1998**, *9* (6),
ahard, D.; Turner, K.;
sens, L.; De Clercq, E.;
Balzarini, J. Phosphoramidate Derivatives of D4T as Inhibitors of HIV: The Effect of Amino Acid Variation. *Antiviral Res.* **1997**, *35* (3), 195−204.
(25) Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; Zuccotto, F.;
Balzarini, J. Design and Synthesis of
4T-MP Prodrugs Expressing High
lture: Structural Determinants for in
Chem. **1999**, *42* (20), 4122−4128.
C.; Ballatore, C.; Srinivasan, S.; De Clercq, E.; Balzarini, J. Enhancing the Aqueous Solubility of D4T-Based Phosphoramidate Prodrugs. *Bioorg. Med. Chem. Lett.* **2000**, *10* (4), 381−384.
(27) Michalowicz, J.; Duda, W. Phenols - Sources and Toxicity. *Polish J. Environ. Stud.* **2007**, *16* (3), 347−362.

Science is built upon the work of contemporaneous and earlier generations of investigators

"Tower of Truth"

https://doi.org/10.1021/acs.jmedchem.1c01444
J. Med. Chem. 2021, 64, 16425−16449

Science information undergoes rigorous scrutiny

However…

Is Any Incorrect Information Published?

- Yes
- Linus Pauling and Robert B. Corey "A Proposed Structure of the Nucleic Acids"
  - ✓ *National Academy of Sciences* **39**, (1953): 84-97
- See also

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

The nucleic acids, as constituents of living organisms, are comparable in importance to the proteins. There is evidence that they are involved in the processes of cell division and growth, that they participate in the transmission of hereditary characters, and that they are important constituents of viruses. An understanding of the molecular structure of the nucleic acids should be of value in the effort to understand the fundamental phenomena of life.

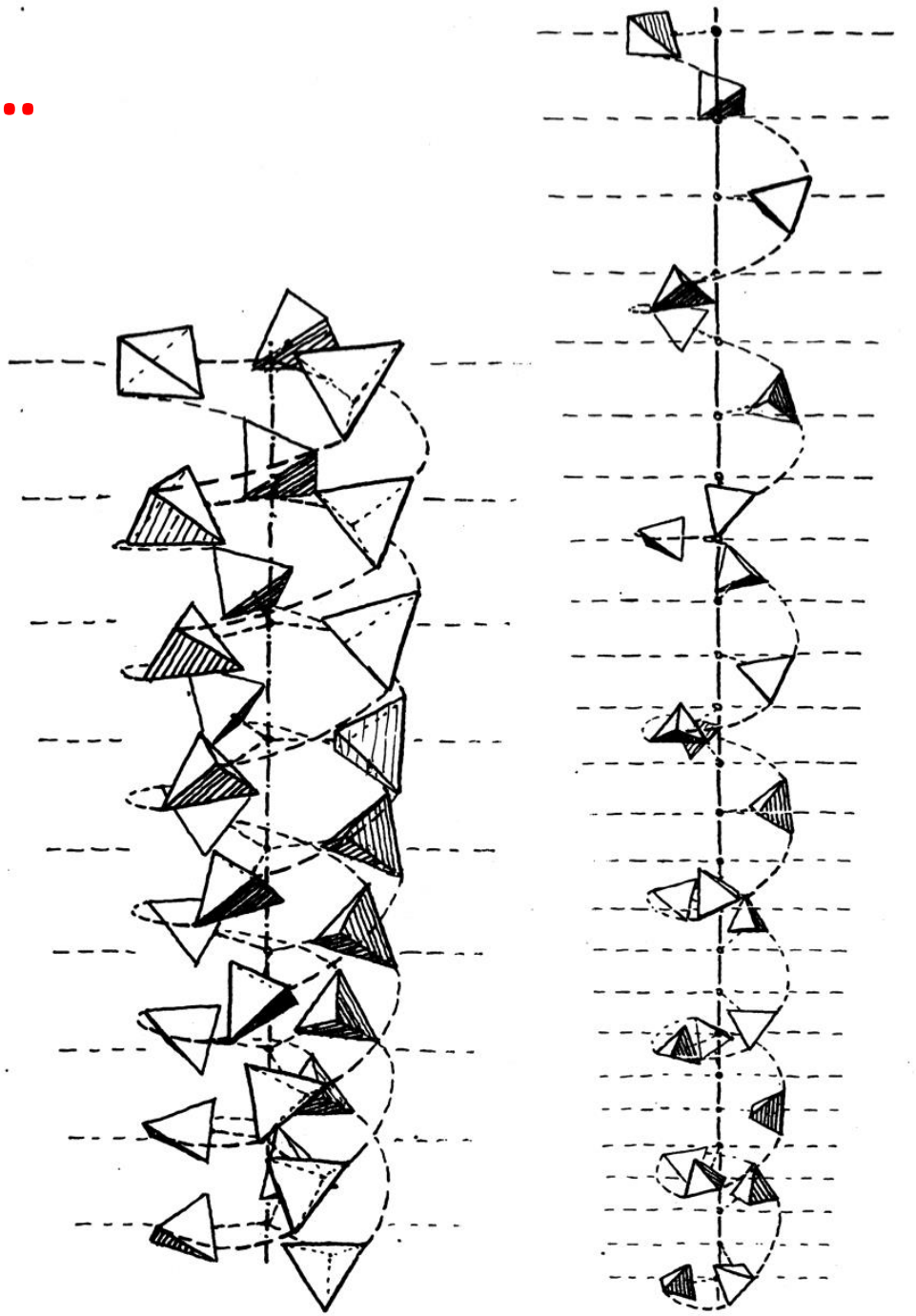
We have now formulated a promising structure for the nucleic acids, by making use of the general principles of molecular structure and the available information about the nucleic acids themselves. The structure is not a vague one, but is precisely predicted; atomic coordinates for the principal atoms are given in table 1. This is the first precisely described structure for the nucleic acids that has been suggested by any investigator. The structure accounts for some of the features of the x-ray photographs; but detailed intensity calculations have not yet been made, and the structure cannot be considered to have been proved to be correct.

The Formulation of the Structure.—Only recently has reasonably complete information been gathered about the chemical nature of the nucleic acids. The nucleic acids are giant molecules, composed of complex units. Each unit consists of a phosphate ion, HPO_4^{--}, a sugar (ribose in the ribonucleic

Sorry Linus…
It's wrong!

Nobel Prize in Chemistry 1954

Nobel Peace Prize 1962



The Correct Structure of DNA

- Four months later (April 25, 1953)…
- J. D. Watson and F. H. C. Crick, *Nature* **171**, (1953), 737-738

Page 1 of 2

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

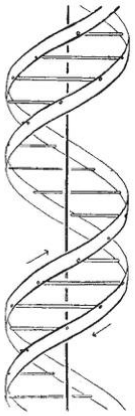
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

note

Page 2 of 2

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. Watson
F. H. C. Crick

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge.
April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

While the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1–3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the internucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through

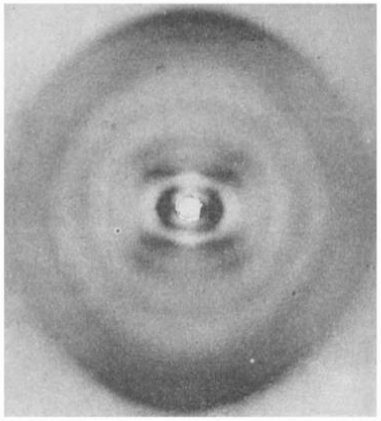


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_0^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to C in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

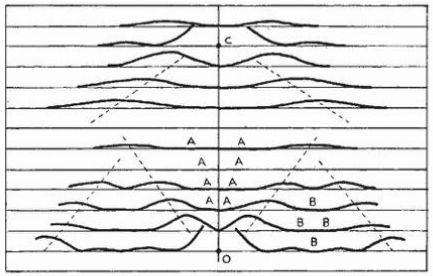
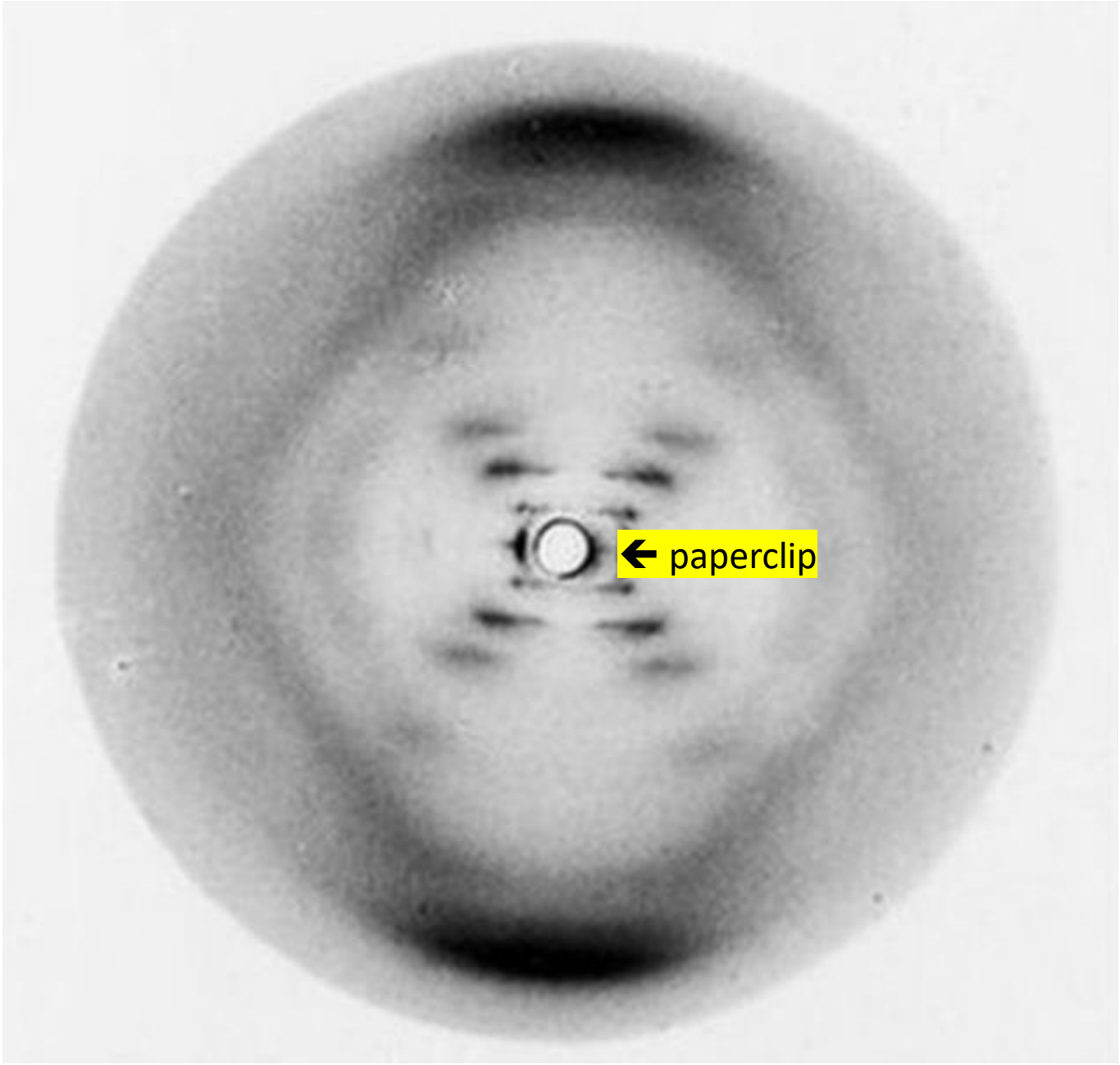


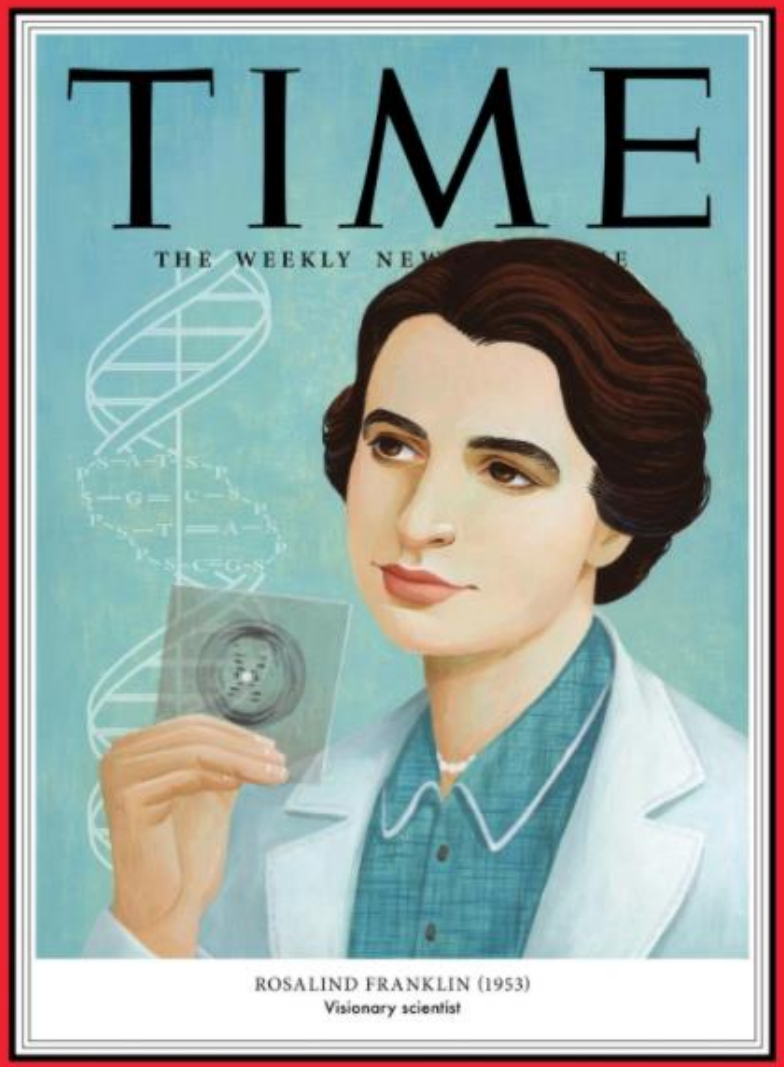
Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About C on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

← About this photo

Rosalind Franklin's X-ray diffraction of DNA Photo #51



Rosalind Franklin
1920-1958



100 “Women of the Year issue”

Age 25

Ph.D. Cambridge University
(physical chemistry)

The Nobel Prize in Physiology or Medicine 1962

Photo from the Nobel Foundation archive.

Francis Harry Compton Crick

Prize share: 1/3

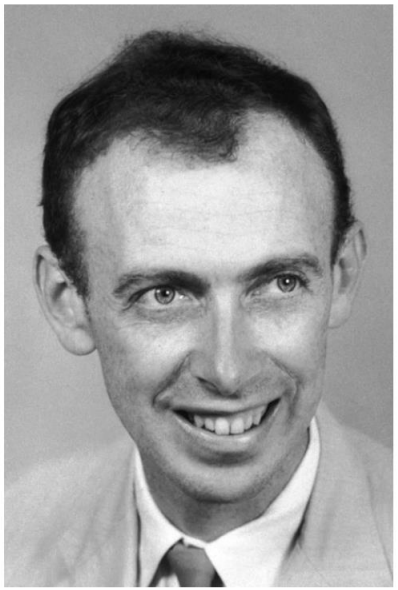


Photo from the Nobel Foundation archive.

James Dewey Watson

Prize share: 1/3

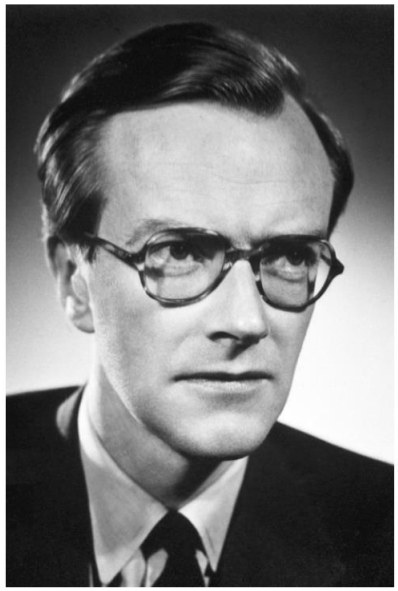


Photo from the Nobel Foundation archive.

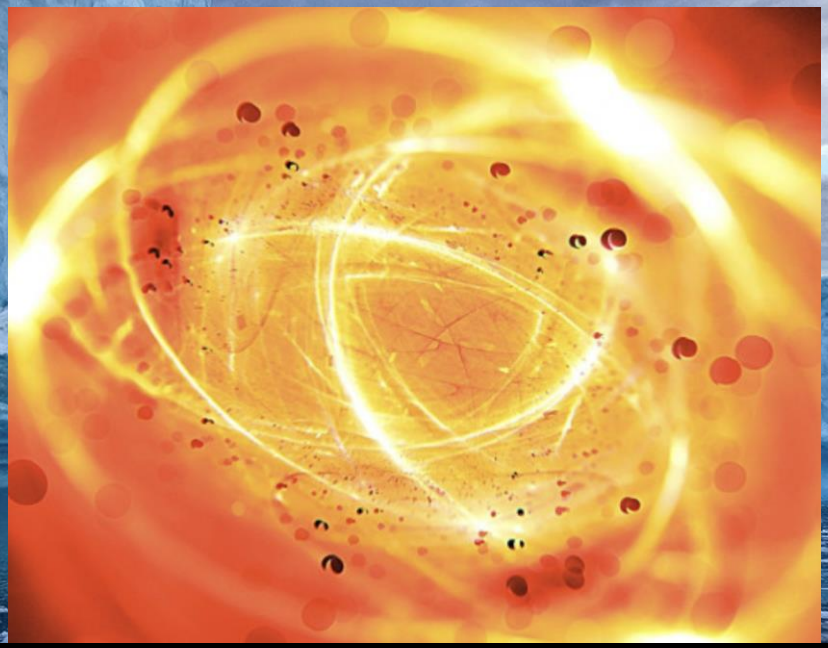
Maurice Hugh Frederick Wilkins

Prize share: 1/3

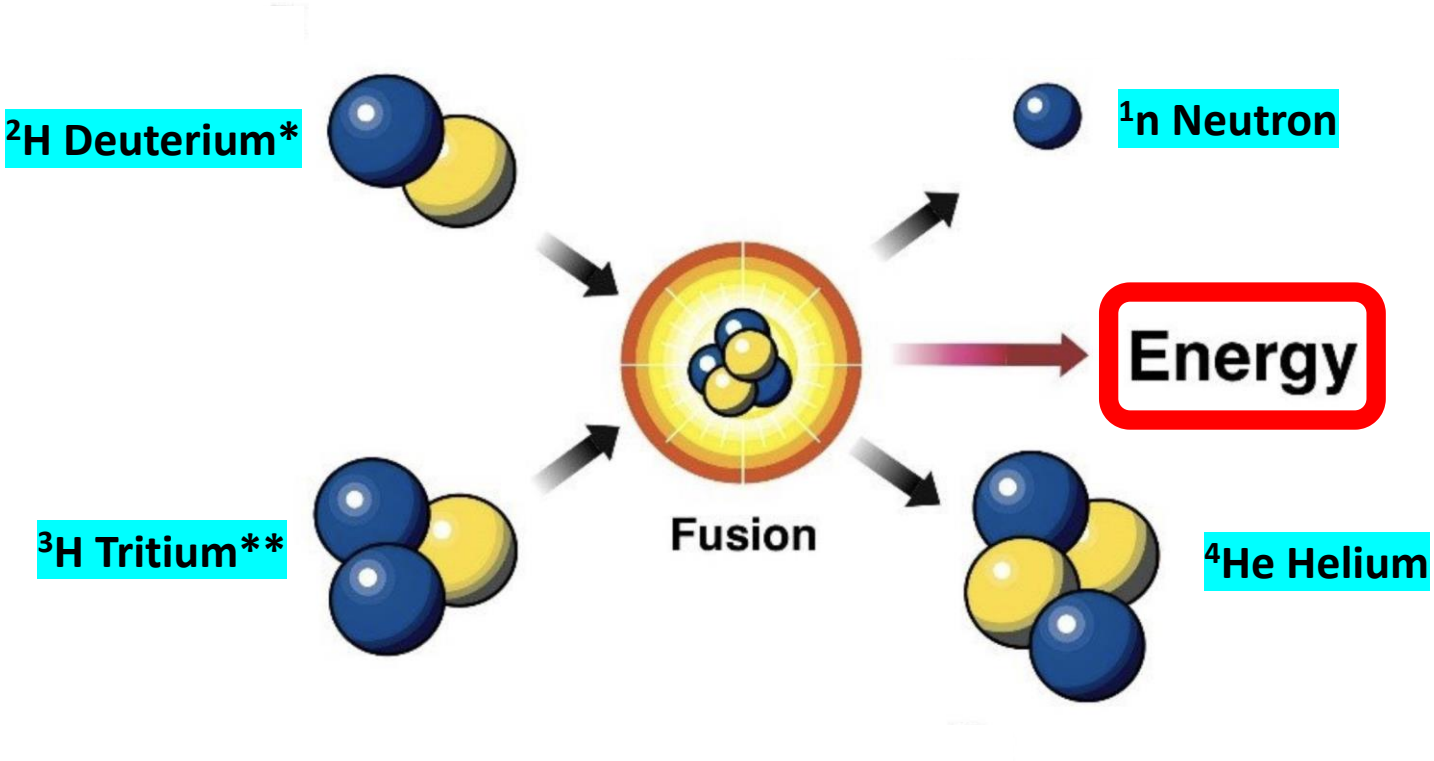
The Nobel Prize in Physiology or Medicine 1962 was awarded jointly to Francis Harry Compton Crick, James Dewey Watson and Maurice Hugh Frederick Wilkins "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material."

Cold Fusion

(Nuclear)



Nuclear Fusion In Our Sun



* ^{2}H occurs naturally on earth; 1 atom ^{2}H to 6420 atoms ^{1}H

** Trace amounts ^{3}H in earth's atmosphere and ground water
^{3}H is radioactive; half-life of 12.3 years

What About Cold Fusion?

- Martin Fleischmann and Stanley Pons held a Press conference—March 23, 1989
  - ✓announced they had accomplished nuclear fusion under normal laboratory conditions
  - ✓public announcements prior to peer-review publication are considered unethical
  - ✓announcement immediately met with great skepticism

What About Cold Fusion? (cont.)

- Subsequently Fleischmann and Pons published: *J. Electroanal. Chem.* **261**, 301-307 (1989)
- Claimed…
  - ✓ "excess heat" had been produced in the experiment
  - ✓ detected neutrons
  - ✓ subsequently errata published in *J. Electroanal. Chem.* **263**, 187 (1989)

What About Cold Fusion? (cont.)

- Millions $ spent on research on cold fusion
- Scientific community now considers the original claims unsupported by the evidence
- Cold fusion is widely considered to be discredited
- Pursuit of the dream continues, see

How Is Erroneous STEM Information Corrected?

- Tends to be self-correcting:
  - ✓replication of experimental procedures affirms conclusions or identifies errors
  - ✓ additional data and study leads to new conclusions
  - ✓ advances in instrumentation leads to more accurate information

Corrections and Retractions

- Corrections of “minor errors” made in subsequent publication (e.g. errata)
- Retractions of publications are made when:
  - ✓ “major” errors made in the original publication
  - ✓ fraud or misconduct has occurred
  - ✓ retractions can be made by the author or editor

Science Misconduct*

Discipline	No. Incidents
Biology and Biomedical Sciences	43
Chemistry **Leo Paquette**	2
Computer Science and Mathematics	2
Philosophy	4
Physics and Engineering	3
Plant Biology	1
Social Sciences	8
Other	4

← Over ~50 years

As reported in Wikipedia
(as of Dec 30, 2021)

Note: 2.6 million papers published in 2018 alone

Leo Paquette

- Author of ~1200 papers and ~17 books
- Plagiarized sections from an unfunded NIH grant application for which he was a reviewer
- No federal funding for 2 years
- Prohibited from serving on Public Health Service Advisory Committees, Boards, or review groups for 10 years

See

More Examples of Science Fraud

- Scott Reuben (US) falsified and fabricated clinical trials data
- Eric Poehlman (US) convicted of grant fraud and falsifying data (went to prison)
- Martin Stone (Ireland) plagiarized in more than 40 publications
- Karl-Theodor zu Guttenberg* (Germany) plagiarized in doctoral dissertation

* German Minister of Defense—forced to resign

How Many "Bad" Papers Published?

- Roughly 10 million STEM papers published from 1970 to 2013 or 12-14 million 1970 to 2020
- "Bad" papers include retractions and papers stemming from science misconduct
- 67 instances of "Bad? papers reported 1970 to 2020 (Wikipedia)
- "Bad" papers = $(67 \times 100)/13 \times 10^6$ = **0.0005%**

Consequences of Misconduct

- Loss of position (fired)
- Loss of reputation (become a pariah)
- Banned from…
  - ✓ publishing in wronged journals
  - ✓ receiving research funding
  - ✓ reviewing publication and grant submissions
- Civil lawsuits
- Criminal prosecution

Science Information for the Ordinary Person

- Nature of STEM journal articles
  - ✓ written for scientists, engineers, etc.
  - ✓ stodgy prose (written in third person)
  - ✓ specialized vocabulary*
  - ✓ highly detailed and frequently long
  - ✓ largely unavailable to the general public
- Consequently—the importance of science writers and reporters

* Like reading a legal document!

Science Information for the Ordinary Person (cont.)

- Translating technical prose into everyday language is hard
  - ✓ simplification leads to inaccuracies
  - ✓ technical vocabulary is challenging to explain
  - ✓ of necessity details must be omitted
- Many people lack basic science knowledge and understanding
  - ✓ there is a difference between astrology and astronomy

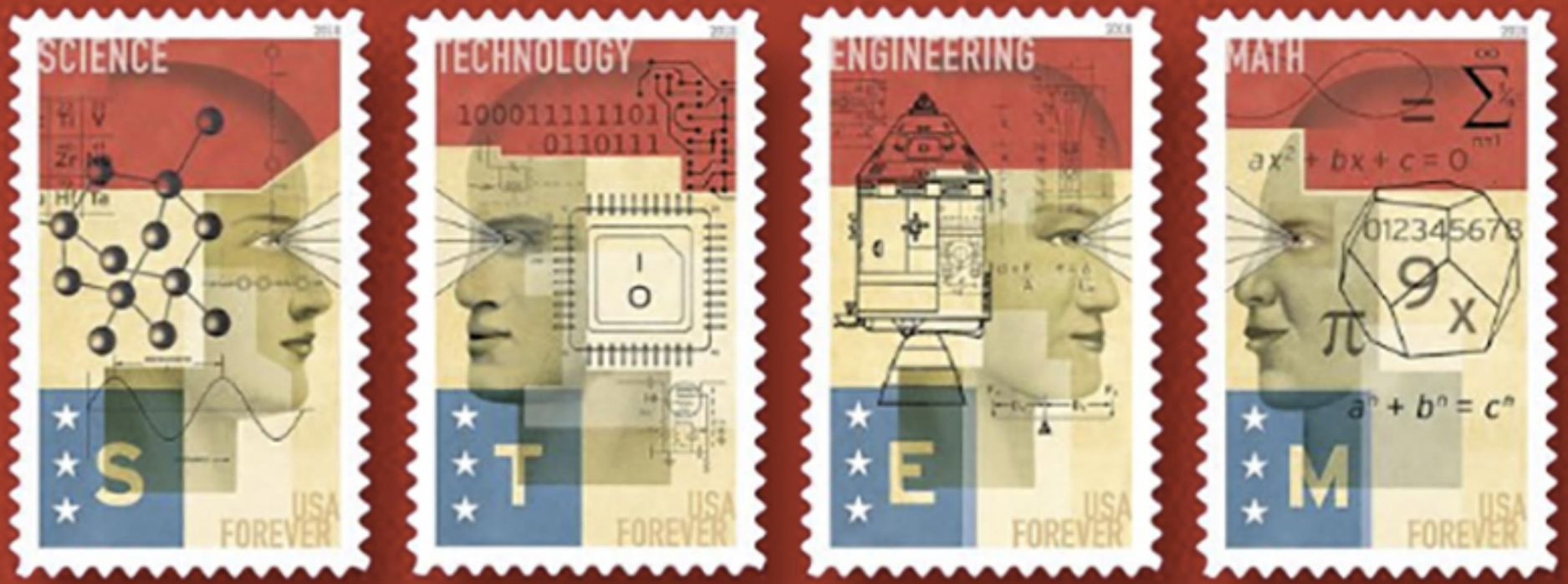
Competition to STEM Information

- Inaccurate or completely false information from:
  - ✓ the Internet (the good, the bad and the ugly)
  - ✓ advertising products and services
  - ✓ celebrity endorsements
  - ✓ newscasters seeking notoriety and market share
  - ✓ politicians (reflecting constituents' views)

An Opinion

- Lack of understanding of science information in the general population is a serious problem—e.g.
  - ✓ COVID-19— deep mistrust of STEM information
  - ✓ mistrust of science information related to climate change
- The nature of science information should be taught in undergraduate introductory science courses
- Also, should be taught in high school science courses

Get off your soapbox, Lorrin!



Thanks for listening!